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THE

SCIENTIFIC PROCEEDINGS

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THE ROYAL DUBLIN SOCIETY.

No. 1.

SEASONAL CHANGES IN THE WATER AND HELEOPLANKTON OF FRESH-WATER PONDS.

By W. R. G. ATKINS

AND

G. T. HARRIS.

[Read October 28. Printed November 13, 1924.]

Introduction.

The British fresh-water plankton has received a considerable amount of study, as indicated in the Wests' monographs, and naturally the seasonal changes have not passed unnoticed. More recently Hodgetts (1921, 1922) and Griffiths (1916, 1923) have reviewed the literature and extended our knowledge. Hodgetts traced the seasonal changes in the water by direct weighings of the total solids obtained by evaporating the filtered water to dryness. He found the lowest concentration to be in April as "a result of the dilution of the pond by the winter and early spring rains." As a rule a low level of the water was accompanied by a high concentration, but to this he found exceptions, for in the autumn water-level and concentration rose together. These facts are interpreted by the present authors in a somewhat different manner, which is more nearly in agreement with the conclusions of Transeau (1916) as regards the enrichment of the water with solutes. From a study of the freezing-point of pond-water Transeau states that the results "indicate that the highest concentrations coincide with the periods of greatest rainfall and higher water-levels, and the periods of low concentration are coincident with low water-levels and drought." His explanation of the low concentrations in drought as being due to the settling of the suspended particles carrying "nearly all the soluble salts with them" cannot be sustained. Hodgetts' pond appears to have contained such relatively large amounts of organic matter (humic substances, etc.) that the relations of the salts to seasonal changes were thereby masked. The water varied in total hardness from 38 to 51 parts per million, of which the permanent hardness amounted only to 5 or 6 parts. It was therefore sufficiently soft for the organic matter to become of importance on

a weight for weight basis; direct determinations showed the salts to be 0·2 to 0·4 of the total. These facts are relevant as regards the seasonal changes found by the present authors.

Whipple (1914), and more recently Pearsall (1923), have pointed out the importance of vertical circulation in lake-water, and its occurrence in the sea has also been dealt with by Atkins (1924). It is considered that salts necessary for algal growth are brought up by such periodical circulation to regions where light penetrates. It has been shown (Atkins, 1923) that lack of phosphate limits the growth of the algal plankton, so that circulatory movement must be effective in replenishing the supply; this permits of renewed growth. The sequence of diatom maxima upon the occurrence of floods has been pointed out by Pearsall (1923). Griffiths (1923), too, has recognised the importance of vertical circulation, and has divided bodies of water into those which at certain seasons have a lower layer deficient in oxygen, and those which are at all times well aerated. The former he finds support Diatoms and Peridinians typically, the latter Chlorophyceae, especially Protococcales.

Pearsall (1921, 1922) has emphasized the importance of the ratio of sodium plus potassium to magnesium plus calcium in distinguishing types of fresh water. Those in which the ratio is high have a predominant Desmid plankton—such water being found in rocky lakes, whereas the silted lakes are richer in magnesium and calcium, and have mainly a Diatom plankton.

Several of the papers mentioned were published while the present research was in progress, but what has been established in them makes a study of the seasonal changes in fresh water all the more necessary. Moreover, work upon the sea has shown that the changes from season to season are small, though of much importance; accordingly it seemed advisable to study fresh water, in which the range of variation is greater.

Observations were begun in 1921, and were continued more systematically from October, 1922, to March, 1924. These include a qualitative study of the free-floating and attached algae—the heleoplankton—together with a few counts by means of the haemocytometer. Lack of time and the difficulty of making any sort of quantitative record for floating masses of Spirogyra, etc., led to the use of the haemocytometer being temporarily abandoned. Analyses of the water were made once or twice a month for over a year as described in a subsequent section.

Methods of collecting and examining the plankton.

The aim of the collections was to ascertain how far it might be possible to correlate the species found with the character of the water, and to study the seasonal changes in the abundance of the constituents of the algal plankton. Up to the present but few counts have been made; as, however, the obtaining of numerical values was the ideal aimed at, nets were never used. Instead, the water was examined under the microscope, without treatment if algae were abundant, but more usually after concentration by means of an electric centrifuge; in this manner the contents were obtained in one-fifth or one-tenth of the original volume of water. The frequencies recorded refer, therefore, to the relative abundance of the organisms, based upon the examination of a number of microscopic fields for each sample. West's symbols *ccc, cc, c, r, rr,* and *rrr* are used.

In addition to the plankton of Staddon reservoir and Borough Farm quarry pond, Antony, the algae which appeared as floating masses in the former, were collected by hand, and examined, as were also, in autumn and winter,

those growing on the cement walls of the reservoir, after the floating masses had disappeared. No such masses were observed in the quarry pond.

General account of the sites and their seasonal changes.

(*a*) *Staddon Reservoir.*—This is an entirely unshaded body of water with a cement wall and a clay bottom, which is overgrown by pond weeds. It is about 22 metres by 7 metres by 1 metre in depth. It has a continual supply of water from a pipe draining a shallow well contaminated by cattle. There is also an intermittent supply from surface drainage. It lies at an altitude of about ninety metres on the east of Plymouth Sound, and is situated in the Staddon Grits, a lower Devonian formation. Samples of water both from the inflow pipe and from the reservoir were examined.

Observations were begun on October 8th, 1921. The water was then quite green, due to large numbers of *Tetraëdron minimum*, with relatively a very few specimens of *Scenedesmus quadricauda*, diatoms, and minute green flagellates. There were no floating masses of algae. On January 7th, 1922, the water had only a slight green tint, and *T. minimum* was the only species present in any quantity. By February 4th the water had become perfectly clear, and the bottom was seen for the first time. This was surprising, since the depth was found to be just one metre. On February 25th the water was still clear. On August 19th the bottom was no longer visible, and the plankton was a pure growth of *T. minimum*. On October 2nd a large amount of *T. minimum* was still present, so as to obscure the bottom, nor could the latter be seen on November 4th. These macroscopic observations are recorded in detail as they give a good indication of the chemical changes taking place and of the variations in the plankton. Observations were interrupted till February 23rd, 1923, when the water was brownish and the bottom invisible, though the previous year it had been clear at this season. By April 3rd the water was clearer and greenish and a little Spirogyra had appeared. On May 1st the water was exceptionally clear, the bottom was visible, and masses of Spirogyra were floating throughout the water. By June 15th, however, the bottom had become obscured, and by the 29th the turbid brownish-green water had masses of Spirogyra and Cladophora floating all over it in patches, amounting roughly to one-fourth of its area. The temperature, 19·2° C., was near the maximum, 19·7° C., which was noted on July 26th, the water still being rather brownish, with some floating weed. By August 24th the amount of weed had diminished considerably, and the water appeared greenish; it was only possible to see into it about six inches on account of a brownish tint which became marked at that depth. On September 26th very little weed was left, and the water was still turbid. By December 17th the water was clear, and the bottom could be seen, as it could also on 31st and on February 5th, 1924. On the 27th the reservoir was still quite clear, and frozen over, but by March 20th it had again become turbid.

(*b*) *Borough Farm Quarry Pond, Antony.*—This is in a disused quarry with precipitous sides, the narrow entrance being closed by a concrete wall. The depth in the centre is unknown, it probably does not exceed 2 metres, but is less near the sides and exit. The pond is about 30 metres square, and the steep banks are topped by bushes and trees, so that the exit and the eastern portion are in shade during summer. It lies in the Antony estate, Cornwall, at an elevation of 50 metres, and is situated in the Upper Devonian Slates, of which its walls are composed. Quite possibly it may have a small supply of spring water, but in very wet weather it receives the drainage from

a roadside ditch. This was seen flowing late in 1923, when the water had been at a low level for some months. The water of the stream was examined when available.

The first pond samples were taken in October, 1922, the water was somewhat brownish, and there were numerous dead leaves. The water-level was high throughout the winter, and the roadside inflow ran, so that there was an overflow at the cement wall of the exit. This ceased in the spring, and on April 21st a plentiful green scum had formed on the water near the exit. It consisted of Chlamydomonas, and had developed rapidly, as none of it had been visible on the 15th. By May 6th the Chlamydomonadine scum, which also contained *Euglena viridis*, had been replaced by Euglena scum, which covered portions of the pond. By June 2nd this green scum extended over the entire pond, save a small area near the exit. By June 9th the Euglena scum had completely disappeared on the surface, though on looking along the water some appeared to be present below the surface. On the 17th no scum was visible, save a little patch at the end remote from the exit, and on the 24th there was in addition a little broken scum near the exit. No scum was found on the 30th. Ducks were never seen on this pond, but moor-hens were found at times. Birds did not, therefore, interfere appreciably with the scum. Counts of the scum-forming organisms are recorded in a later section, as is also a description of a peculiar encystment of the Euglena in the algological notes. During the spring the development of leaves on the trees at the exit end resulted in the shading of about half the area of the pond. Throughout the summer the pond gave an impression of lifelessness and stagnation, and its level fell through evaporation and on account of the water being used for a farm supply-pipe. No floating weeds were observed at any time. The water-level remained low till the winter rains, and, as in 1922, a considerable number of leaves fell into the water.

(*c*) *Plymouth town supply.*—This comes from Burrator Reservoir, Dartmoor. It is a very soft water, largely from a granite catchment area, and was tested for comparison, though it has not as yet been possible to examine the seasonal changes in the plankton.

List of algae.

The following is a list of the algae obtained from the two bodies of water, including the floating filamentous algae found only in Staddon, and the algae associated with them or epiphytic. It also includes the algae growing on the cemented walls of the Staddon reservoir. Those common to both lists are marked with an asterisk, and those found only in Borough Farm quarry pond, Antony, are marked with a cross. In addition the months in which the specimens were collected are indicated, though it is not implied that none were present at other times.

Those algae which were found in the centrifuged water samples in quantity or well distributed through the year are shown in the tables also.

The records are grouped under months, though observations were made more frequently at certain times. In all, the results embody twenty-one collections at Staddon and twenty-three in the quarry.

Flagellatae.

**Euglena viridis* Ehrenb., see table, †*Phacus longicauda* (Ehrenb.) Duj., see table. Total 2.

Myxophyceae (Cyanophyceae).

Hormogoneae.

Lyngyba nana Tilden, Apl., May, Nov., *L. ochraceae* (Kütz.) Thur., May, *Phormidium tenue* (Mengh.) Gom., Apl., May, *Oscillatoria tenuis* Ag., Apl., *O. Agardhii* Gom., Apl., May, *Calothrix parietina* (Näg.) Thur., Oct., *Gleotrichia* sp., Apl.

Coccogoneae.

**Merismopedia glauca* (Ehrenb.) Näg., Apl., **Microcystis aeruginosa* (Kütz.) see table, *Chroococcus minor* (Kütz.) Näg., *C. limneticus* Lemm. Total 11.

Peridinieae (Dinoflagellatae).

†*Peridinium anglicum* G. S. West, see table. Total 1.

Bacillarieae (Diatomeae).

Achnanthes Hungarica Grun., May–Sept., *Amphora ovalis,* Kütz., June, *Cocconeis Placentula* Ehrenb., Apl.–Dec., †*Diatoma elongatum* Ag., Jan., Feb., *Epithemia turgida* (Ehrenb.) Kütz., **Fragilaria capucina* Desmaz, Nov.–May, *Gomphonema constrictum* Ag., usually, *Navicula viridis* Kütz., Apl., *Rhoicosphenia curvata* (Kütz.) Grun., Oct., Nov., **Synedra pulchella* Kütz., see table, *S. Ulna* var *splendens* (Kütz.) V. Heurck, June, *Tabellaria flocculosa* (Roth.) Kütz., *Vanheurckia rhomboides* (Ehrenb.) Bréb. Total 15.

Chlorophyceae.

Isokontae.

(*a*) Protococcales. *Sphaerella lacustris* (Girod.) Wittr., see table, †*Chlamydomonas De Baryana* Gorosch, July, **C. globosa* Snow, see table, **C. Kleinii* Schmidle, Apl., †*Gonium lacustre* G. S. West, see table, †*Pandorina morum* (Mull.) Bory, Oct., June, *Trochiscia aciculifera* (Lagerh.) Hansg., July, **Crucigenia quadrata* Morren, Nov., Dec., **Scenedesmus quadricauda* (Turp.) Bréb., see table, †*S. quad.* var *horridus* Kirchn., June, *S. obliquus* (Turp.) Kütz., Oct., *Elakatothrix gelatinosa* Wille, July, Oct., **Ankistrodesmus falcatus* (Corda) Ralfs, see table, **A. falc.* var *mirabilis* West, Apl., July, Dec., †*A. setigerus* (Schröd.) West, Nov., *Selenastrum gracile* Reinsch., Jan. (West) Sch., Nov., *Kirchneriella obesa* (West) Sch., July to Nov., *Tetraëdron caudatum* (Corda) Hansg., Apl., Oct., **T. minimum* (A. Br.) Hansg., see table, *T. regulare,* Kütz., Apl., **Micractinium radiatum* (Chod.) Wille, see table, †*Lagerheimia genevensis* Chod., see table, **Dictyosphaerium pulchellum* Wood, see table, *Tetraspora gelatinosa* (Vauch.) Desv., Mar., Apl., *Pediastrum Boryanum* (Turp.) Menegh., Apl., May, Nov., *P. tetras* (Ehrenb.) Ralfs, Apl., *P. tetras* var *tetraodon* Smith, Oct. Total 27.

(*b*) Siphonocladiales. *Cladophora glomerata* Kütz., Apl., May, Oct.–Dec. Total 1.

(*c*) Ulotrichales. *Ulothrix subtilis* Kütz., Mar.–May, Sept., Dec., *Hormospora mutabilis* Bréb., *Aphanochaete repens* A. Br., May, Oct. Total 3.

Akontae (Conjugatae).

(*a*) Zygnemaceae. *Spirogyra longata* Kütz., *S. porticalis* (Vauch.) Cleve, *S. varians* (Hass.) Kütz., Apl.–Sept., all three, *Zygnema cricetorum* (Kütz.) Hansg., Apl., one piece only. Total 4.

(*b*) Desmidiaceae. *Closterium acerosum* (Schr.) Ehrenb., Mar., *C. Leibleinii* Kütz., Apl.–Sept., *Cosmarium Botrytis,* Menegh., usually present, *C. crenatum* Ralfs, **C. impressulum* Elfv., usually present, *C. laeve* Rabenh., *C. Meneghenii* Bréb., frequent, *C. punctulatum* Bréb., *C. Regnellii* Wille, frequent, *C. sphalerostichum* Nordst., Aug., **C. Sportella* Bréb., usually present, *C. succisum* West, frequent, *C. undulatum* Corda, *Euastrum binale* var *hians* (Turp.) Ehrenb., June, *Netrium Digitus* Itzs. and Rothe, July, *N. interruptum* (Bréb.) Lütkem., July, *Staurastrum gracile* Ralfs, Nov., *S. Bieneanum* Rabh., var *ellipticum* Wille, June. Total 18.

Stephanokontae.

Oedogoniales. *Oedogonium Rothii* Le Clerc, June, Oct.–Dec., *O. tapeinosporum* Wittr., June, *Bulbochaete* sp., frequent. Total 3.

Heterokontae.

Heterotrichales. *Tribonema bombycina* Derb. and Sol., Apl., Nov. Total 1.

The lists show that, excluding a few doubtful records, the two ponds contain at least eighty-six species, including varieties. Of these the quarry pond contains only twenty-eight, of which seventeen are also found in Staddon reservoir. The absence of floating algae from the quarry pond much reduces the sum total of the list, as these are rich in associated Desmids and Diatoms. No special effort was made to obtain an exhaustive helcoplankton list by searching the precipitous sides of the quarry pond, as the plankton proper was the authors' chief concern.

Tables I and II show which among the algae recorded were the chief constituents of the plankton as obtained by the centrifuge, that is, excluding the filamentous algae and their associates. On considering the tables it is seen that six out of the seven species recorded in the Staddon table are shown in the general list of algae as occurring in the quarry pond, yet only three of them, viz., *Chlamydomonas globosa, Ankistrodesmus falcatuse, A. falcatus* var *mirabilis,* are of sufficient importance to be included in the table for Borough Farm pond. Out of the fourteen in the quarry pond list seven are also found in Staddon reservoir. In the latter Protococcales are almost always predominant, the exception noted being the great development of *Microcystis aeruginosa* in August, 1923, though in August, 1922, the water gave an almost pure gathering of the usual dominant, *Tetraëdron minimum.* These two, together with *Sphaerella lacustris* in October and November, 1923, were the predominant species during the period studied, omitting the development of Spirogyra spp. noted previously.

In the quarry pond no single species is a persistent dominant at all comparable to *T. minimum* in Staddon reservoir. As previously mentioned *Chlamydomonas globosa* and *Euglena viridis* form scums, being present in very great numbers, but these endure for a few weeks only. Several species of Protococcales and Diatoms go to make up the spring maximum. *Peridinium anglicum* abounded in November, 1923, and *Dictyosphaerium pulchellum* was a marked feature of autumn and winter, being accompanied by *Lagerheimia genevensis* and more or less being replaced by *Micractinium radiatum.*

TABLE 1.—STADDON RESERVOIR.

	1922.	1923.											1924.		
MYXOPHYCEAE.	Jan.	Aug.	Oct.	Feb.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
**Microcystis aeruginosa,*	0	0	0	0	0	c	c	c	ccc	c	0	cc	r	0	0	0
CHLOROPHYCEAE, PROTOCOCCALES.																
Sphaerella lacustris, ...	0	0	0	0	0	cc	0	0	0	0	ccc	ccc	cc	0	0	0
**Chlamydomonas globosa,*	0	0	0	cc	r	0	0	0	0	0	0	0	0	0	0	0
**Scenedesmus quadricauda,*	r	0	0	0	c	rr	c	c	0	0	r	0	0	0	0	0
**Ankistrodesmus falcatus,*	0	0	0	0	rr	rr	c	c	0	c	r	0	r	0	0	0
**A. falcatus* var. *mirabilis,*	0	0	0	0	r	r	0	r	r	0	0	r	cc	0	0	0
**Tetraëdron minimum,*	ccc	ccc	ccc	0	ccc	ccc	ccc	c	0	c	ccc	cc	rrr	0	0	rr

TABLE 2.—BOROUGH FARM QUARRY POND.

	1922.	1923.											1924.		
FLAGELLATAE.	Oct.	Jan.	Feb.	Apl.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
**Euglena viridis,*	0	0	0	ccc	ccc	cc	0	0	0	0	0	0	0	0	0
Phacus longicauda, ...	c	r	r	0	0	0	0	c	c	0	0	0	rr	0	0
PERIDINIEAE.															
Peridinium anglicum, ...	r	r	c	c	r	r	r	0	0	r	ccc	c	r	0	r
BACILLARIEAE (DIATOMEAE).															
Diatoma elongatum, ...	0	0	0	0	0	0	r	r	r	rr	0	0	cc	r	0
Nitzschia acicularis (?), ...	0	0	0	0	0	r	0	0	0	0	0	0	0	cc	0
N. longissima (?), ...	0	0	c	0	0	0	0	0	0	0	0	0	0	cc	0
**Synedra pulchella,*	0	r	0	ccc	c	0	0	0	r	r	0	0	0	0	0
CHLOROPHYCEAE, PROTOCOCCALES.															
**Chlamydomonas globosa,*	0	0	cc	ccc	0	0	0	0	0	0	0	cc	cc	r	cc
Gonium lacustre, ...	0	0	0	rr	0	rr	0	r	0	rrr	0	0	0	0	rrr
**Ankistrodesmus falcatus,*	0	r	r	r	0	r	0	0	r	r	0	0	0	cc	0
**A. falcatus* var. *mirabilis,*	0	r	c	0	0	0	0	r	r	0	0	0	r	0	0
**Micractinium radiatum,*	r	r	r	ccc	cc	c	r	r	0	rr	0	c	cc	ccc	ccc
Lagherheimia genevensis, ...	c	c	c	cc	cc	c	0	0	r	r	r	cc	cc	cc	r
**Dictyosphaerium pulchellum,*	ccc	0	0	0	0	r	r	r	r	rr	cc	cc	cc	cc	r

Haemocytometer counts.

The following values were obtained for Staddon reservoir :—

TABLE 3.

Species.	Numbers per c.c. Date—1/5/'23.	30/5/'23.	15/6/'23.
Tetraëdron minimum, ...	8,000	1,600	1,060
Ankistrodesmus falcatus, ...	500	120	—
Small flagellates, ...	13,000	230	3,700
Fields counted, ...	15	53	24

It may be remarked that though May and June have records for *T. minimum,* which are rightly marked *ccc,* yet the June value is only one-eighth of that found early in May. This emphasizes the need for quantitative results. In the Borough Farm quarry pond the diatom *Synedra pulchella* Kütz., was present on April 15th to the number of 16,000 per c.c., on 21st twelve fields gave an average of 30,000 per c.c., and on 24th averages of ten fields each gave 27,000 and 32,000 per c.c. in two samples from different places; it may be remarked that the 32,000 value was obtained at the spot which had given 30,000 on the 21st. The enormous numbers of flagellates constituting the scum is shown by the count for the well-mixed contents of a bottle dipped near the surface on April 21st, viz., *Euglena viridis,* 46,000 per c.c., and *Chlamydomonas globosa* Snow, 5,800,000 per c.c. Even higher values have been obtained by Naumann (1919), who recorded 40 million per c.c. for an unidentified minute green alga, 10 million per c.c. for *Ankistrodesmus falcatus* var *spirilliformis,* and 8 million for *Dactylococcus infusionum.*

Algological Notes.

In the course of the periodical examinations of the two ponds certain unusual forms and rare algae were observed, of which the following may find mention :—

(*a*) *Staddon Reservoir.*—May 30th. *Spirogyra porticalis* (Vauch.) Cleve. The spiral chloroplasts in some of the filaments had lost their characteristic form and filled the cell with a plain parietal cylinder, though the pyrenoids still maintained the spiral disposition. This has also been observed in other species of Spirogyra, but very rarely. It may be explained by Kolkwitz's statement that the chloroplasts grow in length and their edges become fused by pressure. The chloroplasts of both male and female gametes disintegrate during conjugation.

Spirogyra varians (Hass.) Kütz. Several instances were observed of the formation of twin zygospores in one gametangium, an oval and a small spherical one.

Elakatothrix gelatinosa Wille, individuals $4\mu \times 11\mu$. This alga is very rare; one colony was found in the collection of July 26th and a number on October 15th. Its recorded distribution is curious; G. S. West recorded it from Wastwater, G. Smith from one of the Wisconsin lakes; it has also been recorded from Norway, and by Millard Griffiths from a pool in

a clay-pit at Birtley, Durham. West gives a curve for this species in Wastwater, with a maximum late in November and early in December.

Sphaerella lacustris (Girod.) With. On October 29th the centrifuged water gave as deposit a pure gathering of this alga, and nearly pure on November 19th and 27th. Many of the individuals were found to be dividing, the pyrenoids participating in the division.

(*b*) *Borough Farm Quarry Pond.—Lagerheimia genevensis* Chodat was found in January, 1923, with the infusorian *Salpingoeca napiformis* Francé epiphytic on many specimens. These present a curious appearance, as the goblet-shaped infusorian is attached to one side of the much smaller alga. The latter appears to include the variety *subglobosa* Lemm. and a range of forms intermediate between it and the type. Autospores of *L. genevensis* were a marked feature of some of the gatherings, and varied in shape from elliptical to globose. On May 7th *L. genevensis* was plentiful, including an irregular form with five spines not observed in other collections.

The gathering of April 21st, taken when the Euglena-Chlamydomonas scum was on the water, had a considerable quantity of a transparent cellular structure. In some of the cells encysted Euglenas were observed, and in vacant cells the cast off flagellum was clearly seen. There is thus no doubt that the cellular sheet was formed by the encystment of adjacent Euglenas. The persistence of the flagellum seems remarkable in view of its supposed delicate nature; it is possible, however, that what was observed was the impression left by the flagellum upon the substance of the cyst secreted round it. The authors are not aware of this type of encystment, to form a sheet of cells, having been described previously.

Examination of the water.

The observations made were:—

1. Clearness of the water as judged by visibility of the bottom; the results have been given in the section dealing with the macroscopic seasonal changes.

2. Tint of the water as measured against a picric acid solution in a Hehner cylinder. It was found convenient to use the solution prepared for the colorimetric determination of silica, so the tint is given, quite arbitrarily of course, as milligrams of silica per litre, viz., parts per million.

3. Temperature at a depth of about 25 cms., or in sample bottle when water could not be reached.

4. Monthly mean values for the length of the day in hours, sunrise to sunset. These were obtained from the Meteorological Reports for England, S.W. area, and are plotted for the 15th of the month in the graphs.

5. Monthly mean values for the hours of sunshine daily, recorded for England, S.W. area.

6. Hydrogen ion concentration determined at the water's edge or immediately upon reaching the laboratory, using a completely filled bottle. Clark and Lub's buffer mixtures and indicator series were used, taking one drop of indicator adjusted to the middle of its range, to each cubic centimetre of water. For the more alkaline waters A. Cohen's (1922) xylenol blue was also found useful; it gives its colour changes about *pH* 0·2 lower than thymol blue, to which it is similar, but it can be used twice as dilute. The milk bottles of green glass with porcelain stopper, rubber ring and clamp, as customarily used for sea-water samples, were found satisfactory. Bottles with ground-glass stoppers were never used, as the roughened portion gives off alkali.

7. The electrical conductivity was measured by a Kohlrausch bridge reading ohms directly. The Hamburgher cells used had constants close to 1·0, as ascertained by means of a N/100 potassium chloride solution, which at 0° has a conductivity 0·00078. For waters of low conductivity a Kohlrausch cell was sometimes used as its constant was smaller, about 0·1. All measurements were made at 0°, as being the most convenient constant temperature.

A study of the conductivity gives a convenient way of tracing the changes in electrolytes, but is only strictly quantitative if the proportions of the constituents are the same in the various samples, since the migration rates of the ions are not all the same. The principal change observed is, however, one in the amount of the bicarbonates of magnesium and calcium, and for this the method is satisfactory. Furthermore, Vanossi (1922) has shown that when the figure for total solids of filtered potable well and river waters dried at 105° C. lies between 0·1 and 2·0 grams per litre—a very wide range—the conductivity is closely proportional to the total solids. Kolthoff (1922), too, has given constants for calculating the total solids of drinking water from the conductivity.

8. Phosphate was measured by the coeruleo-molybdic colorimetric method of Denigès (1920, '21) as slightly modified by Florentin (1921), using Hehner tubes. The details of the method have recently been given by Atkins (1923). Results have been expressed conventionally as P_2O_5 either in milligrams per litre (p.p.m.) or per cubic metre. These may be converted into values for the phosphate radicle, PO_4, by multiplying by the factor 1·338, which is very approximately $\frac{4}{3}$

9. The soluble silicate, reckoned as silica, was determined colorimetrically by the method of Diénert and Wandenbulcke (1923) which has already been quoted (Atkins, 1923). In the case of water having a tint, as almost all natural water from ponds has, this was measured by comparison with the standard picric acid solution, in terms of silica. Thus, if 100 c.c. of the sample had a tint matched by picric acid solution corresponding to 0·30 mg. of SiO_2 per litre, and if the water plus reagents when compared against a solution of picric acid equivalent to 2·0 mg. per litre gave as result 1·85 mg., the value corrected for tint is obviously 1·55 mg. If, however, the concentration were over 2·0 mg. the depth of the sample looked through would be less than that of 100 c.c., say 80 c.c., and so only $0·3 \times 0·8$, viz., 0·24 should be subtracted as the correction for tint. It may be added that the lighter tints could be matched satisfactorily with the standards, but the tint of the deeper ones was rather too brown. They were matched, however, to appear equally illuminated, as this approximation appeared better than nothing, and it was important not to introduce a fresh determination into the series, the work involved being already heavy.

10. Oxygen absorption was measured by the acid permanganate method given by the Public Health Association of the U.S.A. (1920). A few measurements only were made as time was not available, but further work seems desirable, especially in connexion with the study of down grade processes and stagnation.

11. Oxygen content was not determined directly, but it is known to be high where photosynthetic activity is great. Since saturated calcium bicarbonate solution in equilibrium with air containing about 3 parts per 10,000 of carbon dioxide is close to *pH*8·1, any *pH* value over this shows that photosynthesis exceeds respiration, and may be taken to denote approximate saturation with oxygen; higher values often accompany supersaturation. Conversely, values below *pH*8·1 denote an excess of carbon dioxide above the amount in equilibrium with that of the air, so that a value such as *pH*7·6 denotes a considerable excess, probably accompanied by a reduced percentage of oxygen. With waters poor

in calcium the bicarbonate equilibrium point is at a lower *pH* value. M'Clendon (1917) has given a useful series of graphs connecting bicarbonate concentration and carbon dioxide equilibrium.

The seasonal changes in the water.

The results obtained are shown in the graphs, save that the tint measurements have been omitted from the already rather crowded figures.

It may be seen that, in both ponds, the *pH* value rises in spring as length of day and sunshine increase, the maxima being reached early in April. During this time the temperature is low, and may be rising, as in 1923, or falling for a part of the time, as in 1924. In both ponds the maximum *pH* values are followed by a fall, though the temperature is rising. It is clear, therefore, that temperature changes do not control the algal growth and activity of these ponds, though they have probably a considerable secondary effect in the warming of the upper layers, so that mixing of the water becomes less, and stagnation tends to ensue.

Following the vernal maxima, *pH*8·4 in Staddon reservoir, and *pH*8·7 in Borough Farm quarry pond, there are depressions with minima *pH*7·8 in Staddon in June and *pH*7·2 in the quarry pond in August. These minima are periods of stagnation. The Staddon reservoir, which receives a steady inflow and occasional surface water, soon shows signs of renewed activity, and the water becomes increasingly more alkaline, till late in September *pH*9·1 is reached; this is followed by a rapid fall in winter, and a rise to *pH*8·45 in March. Thus, the *pH* range observed is from *pH*9·3 in August, 1922, to *pH*7·6 in November, and *pH*9·1 in September, 1923, to *pH*7·6 in November, with vernal risings in 1923 and 1924 to *pH*8·4 or slightly over.

The quarry pond, however, which is largely shaded by trees and only receives a visible water supply in wet weather, remains stagnant till the autumn rains replenish it, and its alkalinity then rises to *pH*7·8, for algal activity is renewed and the inflowing water is well aerated. Not till February, however, is there a sharp rise, showing the predominance of photosynthetic effects.

In both ponds the electrical conductivity varies in an inverse manner with the *pH* changes. This appears to be due to the progressive diminution in bicarbonates according as the carbonic acid resulting from their dissociation is used up by the algae. The equilibrium being thus upset, a portion of the bicarbonates is precipitated as the less soluble carbonates of calcium and magnesium, and a further proportion dissociates. The water becomes more and more alkaline while this is in progress till *pH*9·0 is reached. This is the value for pure calcite in saturated solution and boiled to decompose bicarbonates. The fact that higher values, up to *pH*9·7, have been observed under conditions of intense photosynthesis is due to the presence of magnesium salts, for the *pH* value of a saturated solution of magnesium carbonate is close to *pH*10, a fact probably not unconnected with the ease with which it is further hydrolysed to give the hydroxide. This biological precipitation of calcium carbonate must be of considerable importance in geology.

It will be recognised that the combination of *pH* and conductivity measurements given here explains the discrepancies between the conclusions of Hodgetts and Transeau. Hodgetts found low values for total solids in spring, due doubtless to a smaller amount of organic matter, to the precipitation of carbonate and to the using up of other electrolytes by the plants in the course of their development. The softness of the water in the pond he studied

(it varied from 38 to 51 parts per million total hardness, with permanent hardness 5 to 6 p.p.m.) results in the organic matter playing the more considerable part; increase in organic matter in summer is due to conditions of stagnation, which also result in a greater amount of carbonates being dissolved. Transeau appears to have studied a harder water, in which the changes more closely resemble those described here. The decrease found in the freezing-point depressions as recorded by Transeau for spring are quite parallel to

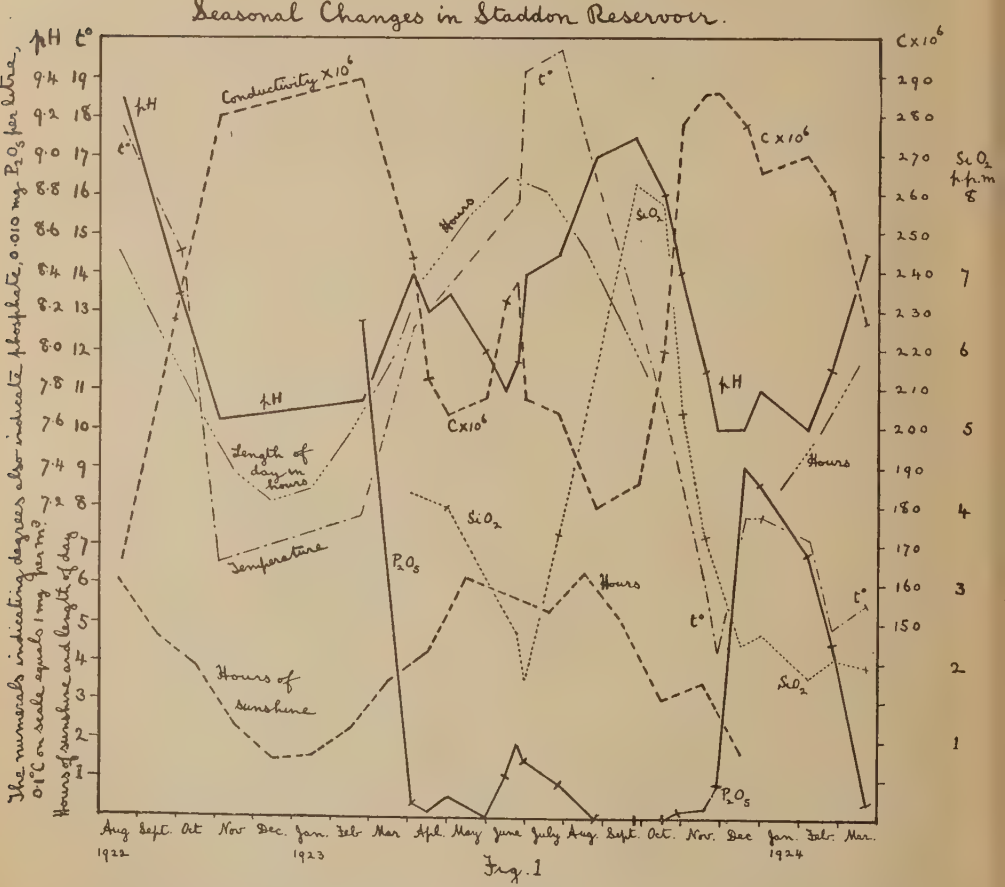
Seasonal Changes in Staddon Reservoir.
pH
t°
C x 10⁶
Conductivity x 10⁶
Hours
Length of day in Hours
Temperature
Hours of sunshine
P₂O₅
SiO₂
The numerals indicating degrees absorb indicate phosphate, 0·010 mg. P₂O₅ per litre, 0·1°C on scale equals 1 mg. per m³, Hours of sunshine and length of day
Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apl. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
1922
1923
1924

Fig. 1

the decrease in conductivity noted by the authors. Precipitation of carbonates and absorption of salts by the developing plankton account for both. Drew (1914) gives records of the changes in total hardness of twelve ponds from January to May, 1913. His results are quite in keeping with the explanation given by the present authors.

It seems that a fall in spring is usual in all ponds containing water which is of any marked hardness. During the whole year there was but little change

in the conductivity of the spring water supplying Staddon reservoir, and the *pH* value, too, was remarkably constant for the freshly drawn water. Loss of carbon dioxide, however, soon produced a noticeable increase in alkalinity.

On turning to the table giving the plankton records for Staddon, it is not altogether easy to correlate them with the changes in the water. Thus *T. minimum* is shown as being abundant very generally, though, as pointed out in the haemocytometer section of this paper, the *ccc* symbol may denote very varying degrees of abundance. In a general way, however, the changes may be correlated with the *pH* variations, especially when it is remembered that during the highly alkaline summer period there were extensive masses of floating algae. The abrupt cessation of the spring rise seems to be connected

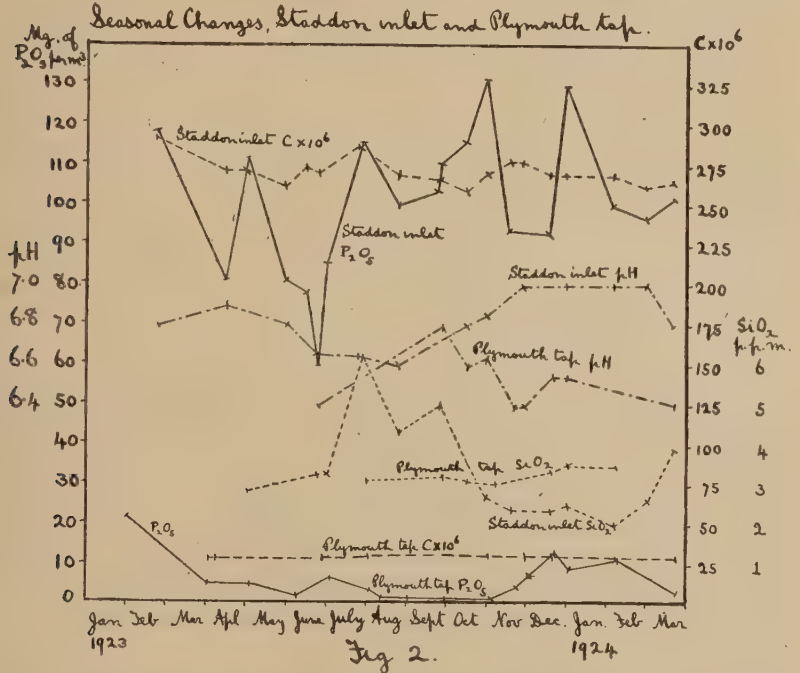

Fig 2.

with the exhaustion of the phosphate supply, which may be seen to have fallen from the relatively high winter value to what is practically zero in April. The stagnation period in June is accompanied by a rise in phosphate, but this is quickly used up again, and there is a renewed outburst of photosynthetic activity. There is, however, all the time a small inflow of spring water which is always rich in phosphates, as shown in fig. 2. The concentration varies considerably, apparently according to the extent to which cattle contaminate the spring.

It is not at all obvious how it comes about that during the winter the Staddon reservoir suddenly becomes filled with clear water, or the water in it clears, and is found to be very rich in phosphates. One would expect the surface drainage to introduce a turbidity, if the phosphate were to be accounted

for by the washing in of matter rich in this constituent. The rate of increase, too, is puzzling. On November 19th phosphate equivalent to 3 mg. of P_2O_5 per cubic metre was found; on the 27th there was 9 mg., but by December 17th the water had become perfectly clear and the concentration had risen to 91 mg. It may be significant perhaps that on the 27th there was ice, below which the water was at 4·3° C., so it is possible that heavy mortality among organisms may have resulted in a sudden liberation of phosphate. This, however, is a pure speculation. As shown in fig. 1 a fall then set in, so that by March 20th, 1924, only 4 mg. was found. Since this reservoir is only one metre in depth, it does not appear that mixing with deeper water richer in phosphate is at all helpful as an explanation. During the critical period, November 19th to December 17th, there were only minor changes in *pH* and in conductivity, but the tint of the water fell from 0·20 to 0·12 or less, for the latter value is at about the minor limit of the estimation. The highest value found for tint was 0·66, in September.

As regards the production of phosphate through its liberation from vegetable plankton, the following observations are of interest:—Water samples were taken from the surface of Staddon reservoir and, by means of a pump, from the bottom at the north end, where the depth is somewhat over one metre. The bottom sample was found to be much the richer in plankton. When taken on October 2nd, 1922, the surface sample was at *pH* 8·3 and at the bottom at *pH* 7·9. On storing, however, till early in March, 1923, the phosphate content was equivalent to 91 mg. of P_2O_5 per cubic metre for the surface and to 277 mg. for the bottom. Subsequent work showed clearly that such amounts of phosphate are not found in the water at that season, so it is evident that they arose through the decomposition of the algal cells present.

As may be seen in fig. 1, Staddon reservoir, at the commencement of the silica determinations in April, 1923, contained over 4 p.p.m. of silica. The water was then clearing after being turbid, and was exceptionally clear in May, during which time, and up to the end of June, the silica decreased, though for the greater part of June the water had been turbid. It may be noticed that the silica curve closely follows that for *pH*, and it seems probable that the greater the alkalinity of the water the larger is the amount of silica dissolved by it, provided that silica is available in suspension. It is remarkable that a very low silica value was found on December 17th, when the water had become clear again, and the phosphate content had suddenly risen. The silica recorded here, it should be remembered, is only that in solution. Curiously enough the low silica values of June coincide with the rise observed in phosphates then. Obviously the multiplication of diatoms must remove silica, but diatoms were never found abundantly in the water of this reservoir, though plentiful among the Spirogyra floating in it. The number of diatoms does not seem to have an appreciable effect in reducing the silica content of this water, which was highest in summer just when diatoms were abundant. Silica can accordingly never be a limiting factor for diatoms in this reservoir.

The silica relations are, however, far from being satisfactorily cleared up, for turning to the values given in the graph of fig. 2 for the Staddon supply-pipe it is seen that a slight rise from April to June is followed by a sharp rise in July, the value 6·25 p.p.m. for the supply-pipe being greater than that for the more highly alkaline reservoir, 3·64 p.p.m. It seems that, in this case, the amount of rain may be an important factor, for it may be imagined that silicates only go into solution slowly; whereas the more readily soluble carbonates quickly saturate the soil water, as is shown by the constancy of the values for the electrical conductivity of the supply. This appears to

be borne out by the rise from January to March, 1924, when the weather was dry. It is also probable that temperature changes affect the solubility of the soil silicates and their rate of hydrolysis, resulting in precipitation of silica.

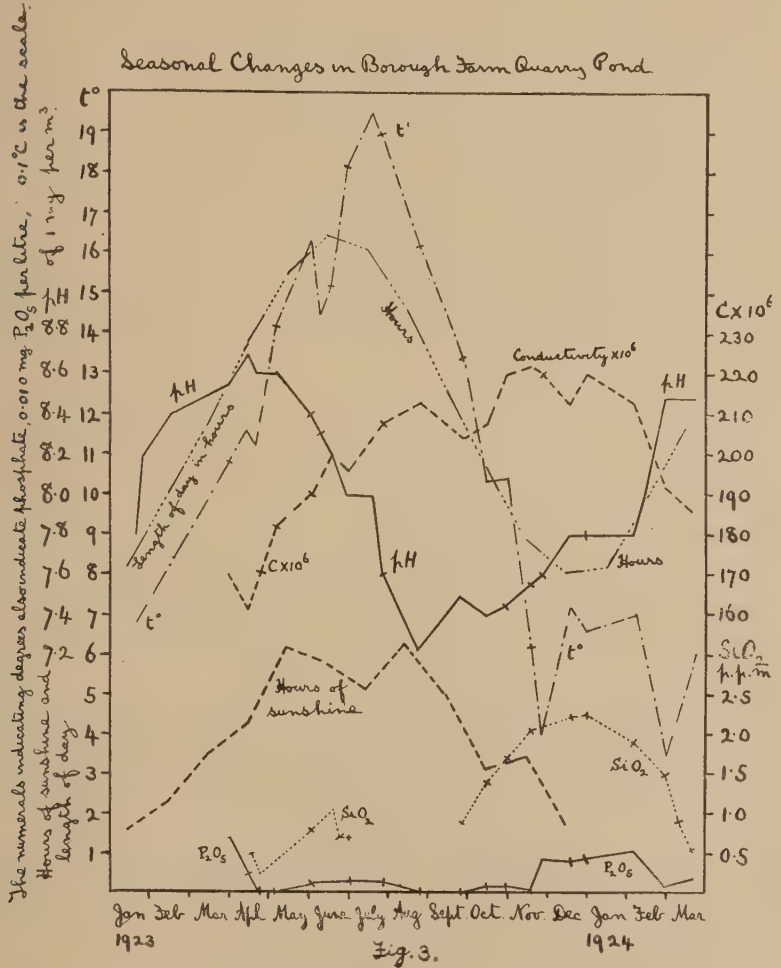
Seasonal Changes in Borough Farm Quarry Pond
The numerals indicating degrees absorbed as phosphate, 0·010 mg P_2O_5 per litre, 0·1°C in the scale of 1 = 1 yr per m^3
Hours of sunshine and length of day
t°
pH
C x 10⁶
Conductivity x 10⁶
Hours
Length of day in hours
Hours of sunshine
SiO_2 p.p.m.
P_2O_5
Jan Feb Mar Apl May June July Aug Sept Oct Nov Dec Jan Feb Mar
1923
1924

Fig. 3.

From what has been said concerning the changes which occur in the reservoir the corresponding changes in the quarry pond may largely be understood from inspection of Fig. 3. Certain points, however, deserve attention. There is, for instance, a much closer correlation here than at Staddon between the alterations in the water and the plankton changes as recorded in the table. This is due to the absence of filamentous algae in floating masses, and of any appreciable amounts of algae on the steep slate sides. The vernal rise in *pH* is closely

associated with the more abundant early spring plankton, and the paucity of life during the summer period of stagnation is quite evident. The autumn plankton and the effect of the inflow of drainage water are both contributory causes to the rise in *pH* to its winter level. As in Staddon, the conductivity curve varies inversely as the *pH* curve, and the exceptional cold of February did not stop, or apparently lessen, the rise of the latter.

The relatively small amount of plankton found in this pond for the summer months seems to be due to the fact that the phosphate supply brought by the inflow which occurs in wet weather, has by then been all used up, and sufficient time has not elapsed for its regeneration from decaying vegetation, and from the animals which grew at the expense of the plankton, either directly or indirectly. It may be remarked that the drop in the silica content occurring in April, 1923, coincided with the increase in *Synedra pulchella* from 16 to 30 thousand per c.c., which has been described previously. This, if not fortuitous, gives an idea of the magnitude of the change 0·28 p.p.m. due to the production of 14 million diatoms per litre. The relation, however, could be ascertained more accurately in a pure culture. The rise in silica accompanying the winter inflow is clearly shown, but the decrease here occurs with increasing alkalinity. Diatoms, however, undoubtedly multiplied during this period of diminution in silica.

It may be mentioned that the roadside stream running into the quarry pond was sampled on December 31st, when it was quite clear, and showed an immeasurably small tint, 0·10 or less. It was found to contain phosphate equivalent to 16 mg. per cubic metre, as against 9 in the pond; the *pH* 7·8 was the same as that of the pond; $C \times 10^6$ was 233, and 220 in the pond, and silica 2·14 mg. per litre, as against 2·26 in the pond. Omitting the correction for tint in the stream the silica value is 2·22, practically identical with the pond value.

Water from the third locality, the Plymouth tap water, is shown in Fig. 2 to be remarkably uniform in its conductivity and in its silica content. Owing to the almost complete absence of calcium and magnesium salts there is no precipitation of bicarbonates. The phosphate content is always low, though it shows a rise in the winter quite comparable to that found to occur in the Borough Farm quarry pond.

Oxygen absorption might be used more than it has been by the writers in studying the enrichment of the water with organic matter, as, even in spring, the quarry pond gave a high figure, 3·34, as against 1·65 for Plymouth tap, and 0·84–1·00 for sea water twenty miles out. The figures given refer to parts of oxygen, by weight, per million of the water examined.

As regards the oxygen content of the water of the ponds, what has been said in §11 of the analytical sub-section of this paper makes it clear that from February to June, 1923, and from February to the end of the observations in 1924, the water of the quarry pond was saturated with oxygen. During the summer, however, the stagnant condition indicated by the low *pH* value 7·2 denotes a great reduction in the oxygen content.

Similarly Staddon reservoir was saturated with oxygen from March till the end of May and from late in June till early in November, 1923, and again from February, 1924. In 1922 saturation ceased about the middle of October. The criterion of saturation taken has been that the *pH* value is at or above *pH* 8·1.

The figures in Table 4 show the changes in Staddon clearly. Thus, even on November 19th the bottom could not be seen, but on December 17th it was visible. The values 0·12, 0·14 denote a negligible tint, and the water could

not with certainty be said to have any colour at all. In the quarry pond the changes are irregular, for the colour is partly due to an earthy turbidity and partly to decaying vegetation, leaves, etc., which fall in. The variations in the tap water appear to be associated with the inflow of peaty water during rainy weather. The Dartmoor rainfall is very heavy, much greater than that of the two coastal ponds.

Table 4.

Tint of water in arbitrary units, colour reaction of parts per million of silica.

Date, 1923–4.	Staddon reservoir.	Quarry pond.	Plymouth tap.
24/9, ...	0·66	0·26	0·32
15/10, ...	0·50	0·40	0·32
29/10, ...	0·30	0·32	0·40
19/11, ...	0·20	0·24	0·34
17/12, ...	0·12	0·36	0·30
31/12, ...	0·14	0·30	0·44
5/2, ...	0·12	0·28	0·26
20/3, ...	0·42	0·34	—

In the results recorded here no mention has been made of the variation in the nitrogenous constituents of the water, the nitrates, nitrites, saline and albuminoid ammonia. Unfortunately it was not possible to carry out these determinations, but it is perhaps legitimate to conclude that they were, in a general way, similar to the changes recorded by Drew (1914) for twelve ponds in the south of England. Drew's analyses may be consulted with profit.

Pearsall (1920) discussing analyses of the water of the English lakes says: "Botanically, their most interesting features are the scarcity of nitrates . . . and the apparent absence of phosphates, which have not been detected at all." The analyses were made upon samples taken during the summer, and the average value for the $-NO_3$ ion was 0·6 p.p.m., the maximum being 2·3 p.p.m. in Esthwaite. The lowest value recorded is 0·4 p.p.m., so anything about or under 0·1 p.p.m. might be regarded as a trace. Yet for phosphates this would be a high value, and estimations are made with certainty to 0·01 p.p.m., while even figures in the third decimal have some significance. It appears, therefore, that, in these lakes also, lack of phosphate limits the increase of the algal plankton, though lack of nitrate may be important in some of them.

Factors influencing algal distribution.

It is at present impossible to account for the observed distribution of fresh-water algae. In some ponds or lakes the commonest forms are absent, though in adjacent and apparently very similar bodies of water they may be abundant. In another pond some rare alga may be the dominant organism. The workers mentioned in the introduction have, however, brought to light certain valuable generalisations, such as Pearsall's, regarding the association of a predominantly Desmid flora with water deficient in calcium and magnesium. This modifies G. S. West's statement that, "The desmid flora in pools and lakes is chiefly

dependent upon the geological formation. It is very poor in lakes situated on the newer formations and correspondingly rich in those on the older formations." West, however, clearly understood that this relation was a secondary one, the primary being that lakes on the older formations were usually poorer in salts.

One cannot, however, make comprehensive assertions about the habitats of large groups, nor, as a rule, is it safe to do so concerning genera. Thus it has been shown by Atkins (1922) that, whereas *Sedum anglicum* is invariably found on acid soil, *S. acre* is only found on alkaline soil, though the two are so similar that they may easily be mistaken, save when in flower. Again, Harris (1920) has demonstrated that the Wests' definition of a Desmid-rich area, one in which 150–200 (or even 300) species can be found in more or less abundance, is amply satisfied by Dartmoor, with 290 species, 92 varieties, and about 30 forms. It is a granite area, with acid soil and water very low in salts. But East Devon, with 287 species, 107 varieties, and 30 forms, also comes within the scope of the Wests' definition, yet it is a Triassic lowland area, with no peat deposits, moderate rainfall, and bogs unimportant both in depth and extent. One is reduced, therefore, to considering algae species by species, and it must be recognised that even species may often have a very wide range, though most abundant under certain conditions. Indeed the correlation between physical and chemical conditions of the habitat and the abundance of any particular alga cannot be properly carried out unless its quantity can be measured either by weight or by counting the number in a definite volume of water.

In seeking to explain the presence or absence of algae in any body of water due regard must be paid to the length of time that it has existed, since many ponds are of very recent origin. Even with this limitation puzzling cases remain. Thus *Tetraëdron minimum,* abundant in Staddon, occurs but very sparsely in the Antony quarry pond. Though only five or six miles apart the ponds differ greatly in plankton and in situation. Griffiths (1922), however, has compared three similar pools in Berkshire—Bulmershe North, Bulmershe South, a few hundred yards away, and Whiteknights' Pool, about a mile west of the latter. They all lie on London clay, a small stream enters the North Pool, and all are supplied mainly by bottom springs. In spite of this the adjacent Bulmershe pools have only four species in common, and South Bulmershe and Whiteknights' only one. Stranger still is the fact that *Ceratium Hirundinella,* a Peridinian, is abundant in all three, yet the three-horned form occurs in North Bulmershe and Whiteknights'; whereas the South pool has the two-horned form only. Through the courtesy of the late Mr. J. Rushbrook and of Mr. G. Hargreaves samples of the water of these pools were obtained during July, 1922, and the electrical conductivities and *pH* values of the South, North, and Whiteknights' pools were found to be respectively 121, 170, and 263 $\times$ 10^{-6} and *pH* 7·0, 7·9, and 7·8 after shaking with air. Thus the Bulmershe pools are definitely softer water than Whiteknights', and, even allowing for a possible decrease before examination, the North and Whiteknights' were the more alkaline.

Many such puzzling distributions could be instanced; probably they are largely due to the mineral and gaseous solutes, and their variation with season and depth, as well as to lack of infection with the several algae. It is, perhaps, profitable to consider the limits of reaction and concentration met with in fresh waters in this country. Saunders (1921) has shown that water from wells in a chalky region is commonly at *pH* 7·1–7·2, rising to 8·25–8·5 in ponds, or up to *pH* 9·0 where photosynthesis was intense. Atkins (1922) records

values from *pH* 6·0 to 8·6 in a variety of situations, and water as acid as *pH* 5·0 has been found in an amber-water bog pool on Dartmoor, and as alkaline as *pH* 9·4 in Kew Lake and 9·3 in Staddon. Values for *pH*, electrical conductivity, phosphate, and silica are also recorded by Atkins (1923, 1, 2). The purest natural water examined was Aberystwyth town supply, $C \times 10^6 = 19$ and *pH* 6·0, followed by Plymouth tap $C \times 10^6 = 26$, *pH* 6·8, ranging upwards to $C \times 10^6 = 164$ and *pH* 9·4 for Kew Lake in July and the values $C \times 10^6 = 270$ common to Basingstoke and Maryfield tap supplies and to Staddon inlet. The purest distilled water obtained here in contact with air has a conductivity $C \times 10^6 = 1$ or less. At the other extreme may be placed slightly brackish water $C \times 10^6 = 2{,}040$, *pH* 7·6 and water from the English Channel, salinity 35·4 per thousand, $C \times 10^6 = 28{,}200$, *pH* 8·2. Thus sea water has a conductivity one thousand times greater than that of the soft water of Plymouth tap and over one hundred times greater than that of a hard supply. Added to this range in concentration, one has to consider the diversity in the composition of the water, both as regards solids and gases; in this connexion the exhaustive monograph of Birge and Juday (1911) should be consulted.

It may be seen, therefore, that there is a very wide range in the conditions prevailing even among fresh waters, and much further work is necessary.

SUMMARY.

1. The seasonal changes in the heleoplankton of two fresh-water ponds have been compared with alterations in the solutes, and it has been shown that in each there is a vernal rise in *pH* followed by a period of stagnation with lowered *pH*. In the quarry pond of Borough Farm, Antony, this condition persisted till the autumn; in Staddon reservoir it was succeeded by a period of high alkalinity lasting till October. These changes are associated with the spring increase in plankton, and the development of masses of floating algae in Staddon reservoir.

2. The electrical conductivity is high when the *pH* value is low, and vice-versa. This may be explained by the precipitation of carbonates of calcium and magnesium, owing to the reduction in the amount of carbonic acid in solution. The reduction in conductivity may in part be due to the removal by the algae of electrolytes necessary for their growth.

3. In both ponds the supply of phosphate is exhausted in spring, and this sets a limit to the further growth of the algae. Staddon pond, however, has an inflow rich in phosphate, which permits of continued growth. Phosphate increases again in winter, partly, it appears, by regeneration, partly by the inflow of water rich in phosphate. A comparison with analyses for the nitrogen compounds found in other ponds makes it highly probable that lack of phosphate rather than lack of nitrate or ammonium salts limits the plankton as a general rule in fresh water, as it does in the sea.

4. The silica content of the two ponds differed considerably, that on the Staddon grits being richer than that on the Upper Devonian slates. The seasonal changes are complex, but it appears that if turbidity due to particles of clay coincides with a high *pH* value much silica, up to 8 p.p.m., may be dissolved. Diatoms may have a small effect in reducing the amount of silica in solution.

5. The tint of the water undergoes more or less irregular changes, partly due to mineral matter, partly to organic substances.

6. The rapid increase in plankton in the spring is associated with the increase in light rather than with increase in temperature; the latter may even fall while the *pH* value continues to rise. The warming of the surface of the water in summer tends to lessen mixing of the water, and so contributes towards stagnation.

7. Oxygen is present to saturation point when the *pH* value is high, but is much reduced when it falls to *pH*7·2.

8. Ponds studied have ranged from *pH*5·0 to *pH*9·4, and electrical conductivity in natural fresh waters from $C \times 10^6 = 19$ to 270, or even 300, at 0° C.

9. In the Staddon pond *Tetraëdron minimum* was usually dominant, and occurred in nearly pure growth; as many as 8,000 per c.c. were found in May. *Microcystis aeruginosa* was very abundant in August, 1923, *T. minimum* having disappeared. In October and November the latter and *Sphaerella lacustris* were abundant. Ninety species of algae, including varieties, are recorded from this pond.

10. In the Borough Farm quarry pond, Antony, only twenty-eight species were found, of which seventeen occur also in Staddon. The pond is at times rich in diatoms, *Synedra pulchella* was found late in April in amounts up to 32,000 per c.c. A scum on the surface at the same period contained *Euglena viridis* 46,000 per c.c., and *Chlamydomonas globosa* 5,800,000 per c.c. as counted by the haemocytometer. *Peridinium anglicum* is also present, being especially abundant in autumn.

11. *Spirogyra porticalis* was observed with laterally fused chloroplasts and *S. varians* with twin zygospores in one gametangium. The rare alga *Elakatothrix gelatinosa* Wille was found in Staddon reservoir. The infusorian *Salpingoeca napiformis* was discovered epiphytic on the unicellular *Lagerheimia genevensis*. A transparent cellular sheet was formed by the encystment of *Euglena viridis* in great numbers.

The authors desire to express their indebtedness to the Director of the Marine Biological Laboratory, Plymouth, for facilities for the carrying out of the analyses of the water.

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No. 2.

THE SYNTHESIS OF UREA FROM CARBON DIOXIDE AND AMMONIA UNDER ATMOSPHERIC PRESSURE.

(Part I.)

By KENNETH C. BAILEY, M.A., D.Sc.,
Lecturer in Chemistry in the University of Dublin.

[Read October 28. Printed November 13, 1924.]

The importance of urea (containing, as it does, almost forty-seven per cent. of nitrogen) as a nitrogenous plant food has made the question of the cheap synthesis of this substance one of some interest. Matignon and Fréjàcques [Ann. Chim. (1922), **17**, 257] have synthesised urea from ammonium carbamate with a yield of over forty per cent. by heating in a sealed vessel at 145° C., the pressure developed being about one hundred atmospheres. The author [Comptes Rendus (1922), **175**, 279] has described experiments devised to test the possibility of synthesising urea from carbon dioxide and ammonia under atmospheric pressure. When a mixture of these gases was passed over various catalysts (zinc oxide, aluminium oxide, thorium oxide, titanium oxide) at temperatures ranging from 110° to 480°, no urea was produced. This is not surprising when the temperature was higher than 140°, as urea begins to decompose at that point, and it was quite evident that the usual catalysts employed to bring about dehydrations were not sufficient to promote the reaction at temperatures lower than 140°.

The synthesis was eventually effected by passing the mixture of carbon dioxide and ammonia through a quartz tube heated to 500° or over, down the centre of which passed a glass tube cooled by a stream of water. Under these circumstances, the reaction

$$CO_2 + 2NH_3 = CON_2H_4 + H_2O$$

took place, and the urea was saved from destruction by being deposited on the cold tube, where it appeared as a white crystalline substance, answering to all the usual reactions for urea. Except at the extremities of the tube, which projected beyond the furnace, where a mixture of ammonium carbonate and carbamate was deposited, the solid on the cold tube consisted of urea and ammonium cyanate. When the product was dissolved in water and the solution evaporated to dryness, the solid obtained melted at 130° C. After one crystallisation from alcohol, needles were obtained, melting at 132° (urea 132·6°).

In the following experiments the tube was heated in an electric furnace (kindly lent by Professor Joly), capable of being maintained at a practically constant temperature for an indefinite period. The temperature of the outside of the quartz tube was estimated by the resistance of a fine platinum wire which passed through the space between it and the central tube of the furnace,

and which had been standardised against a platinum resistance thermometer. The temperature thus recorded is obviously higher than that of the inner surface. The quartz outer tube had an internal diameter of 20 millimetres and a length within the furnace of 1 metre. The glass inner tube had an external diameter of 5 millimetres, the volume of the annular space through which the gases passed being, therefore, approximately 294 c.c. The carbon dioxide was stored over water saturated with the gas, and could be passed from the gas-holder at a constant speed, being dried by bubbling through sulphuric acid. The method adopted for the supply of a regular stream of ammonia was that proposed by Werner [Journ. Chem. Soc. (1918), **113**, 698]. A strong solution of ammonia falls, drop by drop, on granulated caustic soda in a small flask. A stream of almost dry ammonia gas is evolved. In the present experiments ammonia solution of strength about 6N was used, and it was found that, if the flask was first cleared of air, a fairly constant stream of ammonia, corresponding to ninety per cent. of the gas dissolved in the original solution, could be obtained without application of heat.

In the early experiments urea was estimated by Fosse's xanthydrol method, ammonium cyanate being estimated by difference between the urea in the initial product and that present after the solution had been evaporated to dryness. Later, the colorimetric method proposed by K. C. Bailey and D. F. H. Bailey [Proc. R.I.A., June, 1924] was employed for the estimation of cyanate. A series of experiments, performed with the object of finding the relation between the ratio ammonium cyanate : urea and the conditions of experiment, soon proved the futility of such an attempt. The ammonium cyanate is converted into urea during the experiment, and also during the cooling of the furnace before the inner tube can be removed, at a rate which depends on such variable factors as the convection of heat from the quartz tube and the quantity of moisture produced in the experiment. The figures for the proportion of cyanate in the solid product showed, therefore, variation through wide limits, and no deductions of value could be drawn from them.

When it became apparent that urea was the only substance remaining when the solid deposit was dissolved in water and the solution evaporated to dryness, the total urea formed was estimated by simply weighing the residue left after evaporation.

Preliminary experiments showed that the yield of urea, calculated on either component, was increased by using an excess of the other component. If the reaction were performed with the object of manufacturing urea at the lowest possible price, it is clear that the ammonia, which is more expensive than carbon dioxide, must be utilised as fully as possible, and that an excess of the latter gas must be used. A series of experiments (I) was performed to find in what excess carbon dioxide should be used to obtain as great a yield as possible, calculated on the ammonia. When the total volume of the entering gases was about 30 c.c. per min., the temperature of the quartz tube being maintained at 625°, it was found that the percentage yield of urea (calculated on ammonia) increased rapidly with increase of carbon dioxide till the proportion by volume of ammonia to carbon dioxide was about 2 : 5 (theoretical being 2 : 1), and more slowly till the ratio was 2 : 7. Further than this it was impossible to push the experiments, as the total quantity of ammonia in the entering gas became so small that its regular introduction was almost impossible. Two further series of experiments were therefore carried out (II and III), the volumes of entering gases being 60 c.c. per minute in II, and 85 c.c. per minute in III. The yields of urea were, of course, diminished by a more rapid passage of the gases, but curves entirely similar to that obtained in I resulted. It was

found that the maximum yield was obtained when the ratio was 2 : 7 or 8. When the proportion of carbon dioxide was still greater the yield gradually fell off. The explanation, in the opinion of the author, is as follows. The reaction takes place in two stages:—

(1) $CO_2 + NH_3 = (HNCO \rightleftharpoons HOCN) + H_2O$, an interaction which takes place, no doubt, in the neighbourhood of the heated outer tube.

(2) (*a*) $HNCO + NH_3 = HN{=}C\langle^{NH_3}_{O}$ (Urea)[1],

(*b*) $HOCN + NH_3 = NH_4OCN$ (Ammonium Cyanate),

the tautomeric forms of cyanic acid combining with ammonia in the low-temperature zone near the cold tube.

The first stage is promoted by the presence of an excess of carbon dioxide, and this fact is reflected by the rapid rise in yield in the early part of the curve. The fixation of cyanic acid in the second stage is, however, hindered by the dilution of ammonia, and this gradually slows up the increase in yield till, when more than eight times the theoretical quantity of carbon dioxide is present, this effect has become the more important and the yield commences to diminish slowly. There is, then, no advantage in using more than eight volumes, and little in using more than six volumes of carbon dioxide to two of ammonia.

Two further series of experiments (IV and V) were carried out, in which the relation of yield to temperature of the quartz tube was investigated. It was found that when the volume of gases entering per minute was kept constant, the ratio of carbon dioxide to ammonia being also unchanged, the yield increased with rise of temperature as far as 700°, and that the graph obtained by plotting yield against temperature was approximately a straight line. It must not, however, be assumed that this would hold good if the temperature were raised to, say, 800°. With the hot and cold tube at the author's disposal, it was difficult to perform experiments at temperatures higher than 700°, and when these were attempted, the results were not considered sufficiently reliable to be included in the tables appended later. They indicated, however, some falling off in yield. This is not surprising, as dissociation of ammonia into nitrogen and hydrogen occurs at high temperatures.

In some cases cyanuric acid and biuret (to be formed by the polymerisation of cyanic acid and by the action of the latter on urea respectively) were sought in the solid product, but no evidence of their presence was obtained.

Experiments on the use of catalysts were also performed, and it was established that improved yields could be obtained in the presence of such substances as aluminium oxide and thorium oxide. The author hopes, at a future date, to publish a communication on the use of catalysts in this reaction, and on the effect of altering the area of surface of the hot tube and the cold tube respectively.

By collecting the gases which issue from the reaction tube, vaporising the ammonium salts deposited at the exit, and repassing this gaseous mixture several times, the total yield of urea is, of course, increased, and a yield of about fifty per cent. can be thus obtained without difficulty.

[1] For the justification of this formula of urea, the reader should consult "The Chemistry of Urea" by Professor E. A. Werner (Longmans, Green, & Co.).

Experimental.

Series I.—The total volume of entering gases in each experiment was about 30 c.c. per minute. As the rate of flow of each gas was slow, control was difficult, and the rate was much less constant than in the subsequent series. The duration of each experiment was one hour.

Temperature of outer tube.	Volume of gas per min.	Ratio, NH_3/CO_2.	Wt. of urea per hour.	Percentage yield (on ammonia).
624°	29·4 c.c.	2 : 1·1	·1462 grm.	9·6
626°	22·4 „	2 : 3·2	·1308 „	18·9
628°	27·1 „	2 : 4·6	·1557 „	23·6
629°	33·6 „	2 : 7·0	·1499 „	25·0

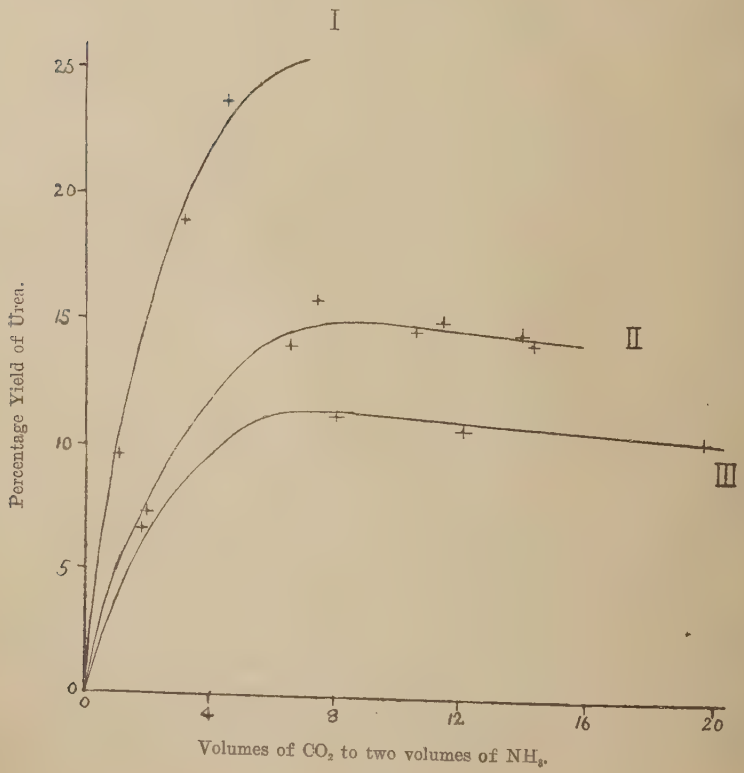

Fig. 1.

SERIES II.—Volume of entering gas, about 60 c.c. per min.

Temperature of outer tube.	Volume of gas per min.	Ratio, NH_3/CO_2.	Wt. of urea per hour.	Percentage yield (on ammonia).
626°	57·3 c.c.	2 : 2·0	·1680 grm.	7·3
625°	62·8 „	2 : 6·6	·1643 „	14·0
624°	64·2 „	2 : 7·5	·1716 „	15·8
626°	58·5 „	2 : 10·6	·1089 „	14·6
628°	57·5 „	2 : 11·5	·1027 „	15·0
623°	59·8 „	2 : 14·0	·0871 „	14·5
626°	58·2 „	2 : 14·3	·0814 „	14·2

SERIES III.—Volume of entering gas, about 85 c.c. per min.

Temperature of outer tube.	Volume of gas per min.	Ratio, NH_3/CO_2.	Wt. of urea per hour.	Percentage yield (on ammonia).
620°	86·3 c.c.	2 : 1·8	·2421 grm.	6·6
625°	87·1 „	2 : 5·8	·2015 „	11·1
625°	82·2 „	2 : 8·1	·1469 „	11·3
620°	85·5 „	2 : 12·1	·1040 „	10·8
625°	83·0 „	2 : 19·7	·0631 „	10·3

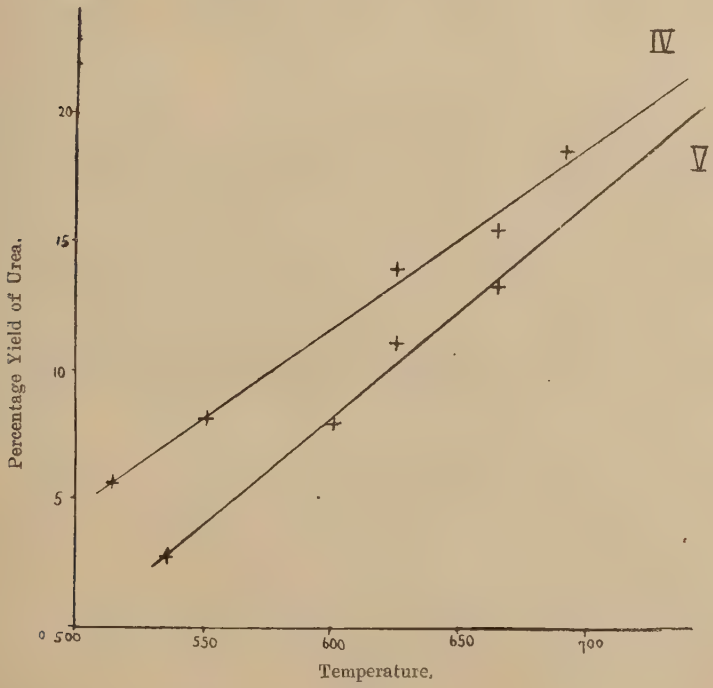

FIG. 2.

SERIES IV.—Volume of entering gases, about 60 c.c. per min., the ratio, NH_3/CO_2, being about 2 : 6. Yield is plotted against temperature of outer tube.

Temperature of outer tube.	Volume of gas per min.	Ratio, NH_3/CO_2.	Wt. of urea per hour.	Percentage yield (on ammonia).
514°	61·3 c.c.	2 : 5·9	·0698 grm.	5·6
550°	63·0 „	2 : 5·8	·1038 „	8·0
625°	62·8 „	2 : 6·6	·1643 „	14·0
665°	63·0 „	2 : 6·4	·1867 „	15·5
691°	61·4 „	2 : 6·4	·2196 „	18·7

SERIES V.—Volume of gas per min., about 85 c.c., the ratio, NH_3/CO_2, being about 2 : 10.

Temperature of outer tube.	Volume of gas per min.	Ratio, NH_3/CO_2.	Wt. of urea per hour.	Percentage yield (on ammonia).
535°	86·0 c.c.	2 : 9·8	·0309 grm.	2·7
600°	84·1 „	2 : 9·1	·0955 „	8·0
625°	84·0 „	2 : 10·0	·1248 „	11·1
665°	83·5 „	2 : 10·1	·1487 „	13·3

SUMMARY.

1. Urea can be synthesised from carbon dioxide and ammonia at atmospheric pressure by passing the mixed gases through the annular space between a quartz tube heated to a temperature higher than 500° C. and an inner water-cooled glass tube.

2. It is advantageous to use the gases in the ratio of two volumes of ammonia to eight of carbon dioxide.

3. The yield of urea increases with temperature to at least 700°.

The author gratefully acknowledges the many helpful suggestions of Professor E. A. Werner and the kindness of Professor J. Joly, in whose laboratory most of the experiments described were performed.

No. 3.

OOGENESIS IN LITHOBIUS FORFICATUS.

By S. D. KING, B.A.,
Assistant to the Professor of Zoology and Comparative Anatomy,
Trinity College, Dublin.

[COMMUNICATED BY PROFESSOR J. BRONTË GATENBY.]

(PLATES I–II.)

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Introduction and previous work.

THE Myriapods, as a group, seem to have been hitherto rather neglected by cytologists, and even morphologists. This is surprising, since their systematic position is rather interesting; although specialised along certain lines, they are nevertheless primitive Arthropods, and one might therefore expect them to be worthy of more attention than they have as yet received.

I am not aware of any papers on gametogenesis in Myriapods, except isolated observations by Fauré-Fremiet, but before giving an account of my investigations it may be as well to mention some work on other groups which is useful for comparison. Hogben, in dealing with the oogenesis of certain insects (5, 6), has described the formation of yolk from nucleolar extrusions, a phenomenon which had not previously been dealt with in a convincing manner by any cytologist, at least in Great Britain. Gatenby later described a similar process of yolk-formation in the Archiannelid Saccocirrus (2). In most animal groups yolk-formation is chiefly due to the activity either of the Golgi apparatus or of the mitochondria, and Hogben suggests that the process may be due to the "interaction of the metabolic functions of plasmosome, mitochondria, and Golgi apparatus," a view which would save us from the assumption that the process of yolk-formation had been independently evolved in different groups. Brambell, in a recent paper on oogenesis in Molluscs (8), gives a useful review of the present position with regard to yolk-formation in animals.

Hogben (6) describes the clumping of the mitochondria to a definitive mass ("yolk-nucleus") in the young oocyte of Libellula, and the subsequent breaking up and scattering of this body, and Gatenby has observed a similar phenomenon in Apanteles (3). Hirschler (4) finds that the definitive mitochondria in Ascidians are developed from several "yolk-nuclei," which are present from the earliest stages in the development of the oocytes, and which are themselves of a mitochondrial nature. These "yolk-nuclei" enlarge, and later become surrounded by peripheral granules, which break away to form the definitive mitochondria, the "yolk-nuclei" themselves retaining their individuality for some time after these have broken away from them. In all these cases the mitochondria are found in a primary concentrated condition, later becoming diffused through the cell; in no case, so far as I know, has a primary diffuse condition been shown to precede concentration.

In Ascidians, Hirschler finds the Golgi apparatus of the oocyte in a primary diffuse condition, later becoming concentrated to a nètlike structure comparable to that of Vertebrates, a fact of considerable interest when we take the systematic position of the Ascidians into consideration. In the great majority of types, however, the apparatus is seen to be eccentric and juxta-nuclear in the young stages of oogenesis; and from this fact, together with recent observations on the Protozoa, we conclude that this is the most primitive arrangement.

Structure of the ovary of Lithobius.

The ovary of Lithobius is a median sac, the walls of which are composed of a germinal epithelium, from which the oocytes and follicle cells are derived. The fact that no mitoses have been observed in the cells of the germinal epithelium points to the conclusion that these cells are young oocytes, not oogonia; and every gradation can be traced from these undifferentiated cells to the larger yolk-laden oocytes ready for fertilisation. As the oocytes proceed with development, they sever their connexion with the wall of the ovary and lie free in its cavity, where they later become surrounded by follicle cells.

Golgi Bodies.

Both Mann-Kopsch and Da Fano material was studied, but the latter gave such very favourable results that it was used almost exclusively. In the youngest cells, found in the germinal epithelium, the Golgi apparatus has the typical juxta-nuclear eccentric position found in most undifferentiated cells (fig. 1); very soon, however, it begins to fragment, at first breaking into a few large masses which move apart. The early stages of the fragmentation of the apparatus can be found in the cells of the germinal epithelium, as in fig. 1 at G_1, where the fact that it has broken into two masses, which have separated, gives it the appearance of a bipolar arrangement, such as one would expect in cells undergoing mitosis. This, however, does not seem to have any special significance, since, although the bipolar arrangement is fairly common in these cells, it is by no means universal, the apparatus often breaking up into several irregularly scattered masses, so it must be regarded as a chance disposition of the Golgi bodies. As the oocyte grows (a fairly rapid process in the early stages) the Golgi bodies appear to increase in volume, and form large irregular masses scattered through the cytoplasm. These gradually break up into smaller masses (fig. 2). The process of fragmentation continues, and a later stage is shown in fig. 3, under a very high magnification. Here the masses are seen to be breaking up into smaller grains. The Golgi bodies thus become smaller and smaller, till, in the early stages of yolk-formation, they are quite unrecognisable, though probably represented by fine granulations in the cytoplasm of the Da Fano fixed oocytes.

Mitochondria.

The behaviour of the mitochondria during oogenesis is rather remarkable. They were studied in material fixed in Flemming-without-acetic and stained in iron hæmatoxylin. In the early oocytes of the germinal epithelium the mitochondria appear as a small number of rather large grey-staining granules scattered irregularly in the cytoplasm (fig. 4). They seem to remain in this condition until the oocyte begins to enlarge, when they collect in a cloud at one side of the cell (fig. 6), having by this time increased somewhat in number. As the oocyte enlarges still more, this cloud seems to fragment, but the mitochondria do not yet spread out evenly through the cytoplasm,

but remain collected in clusters, some of which attain a very regular spherical outline. Figs. 7 and 8 represent oocytes at this stage. In fig. 8 some of the mitochondria are seen to be collected in loose clouds, chiefly round the periphery of the cell, while others are grouped together to form the curious round body at *MX*. These bodies are of varying sizes, and are found in varying numbers (one to four) in different oocytes. The individual mitochondrial granules of which they are composed can usually be clearly made out, at least on the surface. It is remarkable that cells in which all the mitochondria appear to be collected in these clumps are rarely or never found, some of them being almost always scattered in clouds as shown in fig. 8. In material fixed by methods injurious to mitochondria, as, for instance, Bouin's fluid, the scattered clouds are invariably destroyed, while the round clumps resist the fixation, thus resembling the mitosomes of many sperm cells. A comparison of figs. 6 and 8 will make it clear that the mitochondria increase greatly in number during the earlier stages of oogenesis, and it seems probable that the round mitochondrial bodies are simply dense clumps formed by rapid multiplication. The clouds of mitochondria lying loose in the cytoplasm may have been derived from the precocious breaking up of these clumps, but it seems more probable that they represent areas in which the multiplication is not taking place with sufficient rapidity to produce dense masses. In fig. 9 an older oocyte is represented, in which the mitochondria are beginning to scatter through the cytoplasm. At *MX* an early stage in the fragmentation of one of the round masses can be seen, while the irregular clouds of mitochondria at *M* are probably derived in part from the breaking down of other such masses, and in part from the clouds which lay free in the cytoplasm, as described above. As they scatter through the cell the mitochondria tend to become arranged in irregular threads, scattered through the smooth cytoplasm (fig. 10). This appearance is not due to the individual granules running together, but to their arrangement in lines, which have no definite order. In the next stage the mitochondria become evenly distributed through the ground cytoplasm (fig. 11), and do not appear to undergo any further changes.

Nucleolus.

The nucleus of the young oocyte in the germinal epithelium contains a large central nucleolus of irregular shape (fig. 4) lying towards its centre; the chromatin is arranged in a network in the typical way, and around the periphery of the germinal vesicle, in contact with the nuclear membrane, a number of darkly staining bodies can be seen. These are nucleolar in nature, and can be seen budding off from the central nucleolus, whence its irregular shape. These bodies are extruded into the cytoplasm at an early stage, but their subsequent fate has not been determined, as they seem to disappear shortly after leaving the nucleus. The actual passage of these particles to the cytoplasm is very rarely shown, as it probably takes place with considerable rapidity; but the oocyte shown in fig. 5 may represent this stage, since here the central nucleolus remains undivided, while the peripheral granules are passing out into the cytoplasm. The process has generally been completed in oocytes of this size, and the mitochondria have usually collected to a cloud at one side of the cell (fig. 6), whereas in this cell they are still in the primary diffuse condition; it is doubtful, therefore, whether any definite conclusions can be drawn from this figure, since it is taken from an isolated observation and may represent an abnormal oocyte.

The fate of the large central nucleolus can be followed with more certainty than that of these early nucleolar extrusions. At an early stage, as soon as

the young oocyte begins to enlarge, the nucleolus breaks up into a number of pieces, which gives a characteristic rosette-like appearance in the centre of the germinal vesicle (fig. 6). These pieces now enlarge and start a process of budding, by which the number of nucleolar fragments is greatly increased, the ultimate result being the fragmentation of the nucleolus into a large number of very small grains (fig. 9). The budding of the nucleolar fragments can be well seen in fig. 7, which also illustrates the passage of large masses from the germinal vesicle to the cytoplasm. This secondary extrusion of nucleolar matter takes place usually at a later stage than that represented in this figure, when the fragments have become finely divided as in fig. 9, and in all cases the greater part of the extrusion takes place after this condition has been reached; it is not uncommon, however, to find a few larger masses extruded at an earlier stage. The nucleolar grains, on reaching the cytoplasm, increase in number by budding, the grains so formed eventually enlarging into yolk-spheres. Fig. 12, from a Flemming-without-acetic-iron-hæmatoxylin preparation, shows yolk-spheres at all stages of development, while most of the nucleolar grains have been extruded from the nucleus. The extrusion takes place gradually, and not all at one time; and this accounts for the great difference in size of the yolk elements. In the oldest oocytes the cytoplasm becomes quite laden with large round yolk-spheres.

Owing to the great difficulty experienced in obtaining full-grown oocytes, no detailed investigation has been made beyond the stage shown in fig. 12.

The secondary extrusion of nucleolar substance begins much earlier in some oocytes than in others; most generally it takes place at about the time when the mitochondria are scattering to the secondary diffuse condition, but cells such as that represented in fig. 7 show that there can be no connexion between the two events.

Fatty Yolk.

In unstained oocytes fixed in Flemming-without-acetic certain of the yolk-spheres are seen to be blackened by the OsO_4. The origin of this fatty yolk is doubtful, but it may possibly be connected with the Golgi apparatus, although no evidence in support of this theory has been discovered.

Changes in chromophility of the cytoplasm.

This was studied in material fixed by Bouin's fluid, absolute alcohol, and Petrunkewitsch, and stained by Scott's Ehrlich's hæmatoxylin and Biebrich scarlet method. The cytoplasm of the young oocytes of the germinal epithelium is basophil, and this condition persists up to about the time when the mitochondria become scattered, when the cytoplasm becomes amphophil, and gradually changes its staining reactions till it is very strongly oxyphil. The nucleolar yolk-spheres are basophil in the earliest stages, but when they begin to swell they are strongly oxyphil. In an oocyte in the stage shown in fig. 12 the only basophil granules in the cytoplasm are the nucleolar extrusions which have not yet begun to swell. The primary membrane, which forms round the oocyte at a comparatively late stage, is oxyphil from the first, but the follicle cells retain their basophil character throughout oogenesis.

Other cells in the Gonad.

The young oocytes, which have just left the germinal epithelium, can be distinguished by the nucleolus, which is usually in the state represented in fig. 6; but certain cells are found in the ovary which show somewhat different

characteristics. The cytoplasm of these cells has the appearance shown in fig. 13, being loaded with darkly-staining granules. The nucleus differs from that of the oocyte in containing very little nucleolar matter, and one is tempted to conclude that the dark granules in the cytoplasm are formed of extruded nucleolar grains. These cells are found in the follicles, lying side by side with the more typical follicle cells, which resemble them in their nuclei, but differ in having a very clear cytoplasm (fig. 14). An obvious explanation of the differences of these two types of cell is to regard those in which the cytoplasm is loaded with dark-staining granules as the youngest follicle cells, which later use up the granules in secretion, and revert to the more typical cells with clear cytoplasm. The chief difficulty in the way of accepting this view lies in the fact that the two kinds of follicle cells are found side by side in the same follicle; whereas one would expect to find young follicles entirely composed of the first kind of cells and older ones of the second.

Discussion.

The most interesting points about the oogenesis of Lithobius are to be found in the method of yolk-formation, and in the behaviour of the mitochondria. Most of the yolk is formed directly from the nucleolar fragments extruded into the cytoplasm, but the process usually begins after the Golgi apparatus has broken to quite small pieces, and the mitochondria have scattered to the secondary diffuse condition. This seems to support Hogben's view already alluded to; but against this is the fact that it is not uncommon to find a few fairly large yolk spheres in the cytoplasm of cells in which the mitochondria are still in clusters. It seems probable that the formation of yolk by the nucleolus will be found to be general in the Arthropod phylum. Until recently there has been much confusion in descriptions of nucleolar extrusions, which have been very generally accepted as chromatinic; but modern research has shown that in many cases these supposed chromatin emissions are really of a nucleolar nature. Thus the peculiar secondary nuclei, which have been described in many Hymenoptera, and attributed generally to budding of the germinal vesicle, or chromatin emission, have now been shown to originate from the nucleolus. Since these secondary nuclei are known to be active factors in yolk-formation, this supports the view that yolk is generally formed under the influence of the nucleolus in Arthropods, and future research will almost certainly provide us with other instances.

The nature of the peculiar round mitochondrial bodies shown in Figs. 7 and 8 at *MX* is rather doubtful. They remind one at first sight of the so-called "mitosomes" described by Hogben in the oocytes of Libellula, but differ from these in being present in varying numbers in different oocytes, and in not containing all the mitochondria of the cell, some of which remain in scattered clouds. They resemble the yolk nuclei of Hirschler's Ascidians in their varying number, but differ from them fundamentally in other respects. Thus the Ascidian yolk nuclei are mitochondrial bodies, which are present in the earliest oocytes, and give rise to all the definitive mitochondria; the mitochondrial clumps of Lithobius are, on the other hand, secondary, being formed by a concentration and subsequent proliferation of the primarily diffuse mitochondria, and it has been shown that some of the mitochondria remain in scattered clouds throughout oogenesis, and so have no connexion with the round masses. Again, the yolk nuclei of Ascidians are not purely mitochondrial, since they retain their individuality for some time after the scattering of the definitive mitochondria, while the round bodies in the oocyte of Lithobius break up completely when the scattering takes place, so that no trace of their former position can be seen. Thus it seems that the mitochondrial masses of Lithobius are not comparable

either to the yolk nuclei of Hirschler's Ascidians or to the "mitosomes" described by Hogben in Libellula, and it seems most reasonable to regard them as the product of active multiplication of certain groups of mitochondria.

The behaviour of the Golgi apparatus is, as already shown, very ordinary. It is, however, of interest, since it shows that in Lithobius, as in Dytiscus, the only other Arthropod in which the behaviour of the apparatus during oogenesis has been worked out, it takes a minor part, if any, in yolk-formation. In this the Golgi bodies of Lithobius will probably be found to resemble those of Arthropods in general, even as one may expect the importance of the part played by the nucleolus in the yolk-formation of this phylum to be more generally realized as a result of further research.

Summary.

1. Yolk-formation is from nucleolar extrusion, of which two phases can be distinguished. In the first phase the extruded particles are budded off from the central nucleolus, and their fate after extrusion has not been determined; in the second phase the particles are formed by fragmentation of the nucleolus, and after proliferation grow into the definitive yolk spheres. The Golgi bodies and mitochondria do not take any direct share in yolk-formation.

2. The mitochondria are described in a primary diffuse condition, becoming concentrated in the early stages of growth. They then form several clusters, some of which proliferate very rapidly, giving rise to round bodies which are not comparable to the yolk nuclei of Ascidians, or the mitochondrial masses described by Hogben and Gatenby in insect oocytes. Later the mitochondria become evenly distributed through the cytoplasm.

3. The Golgi apparatus behaves in the usual way, being concentrated in the youngest oocytes, and later spreading out through the cytoplasm, breaking into very fine granules.

4. Oocytes and follicle cells are derived from the germinal epithelium of the ovary wall.

DESCRIPTION OF PLATES.

Lettering:—F. Follicle cell nucleus, G. A. Golgi apparatus, G. Granules in cytoplasm, possibly nucleolar, M. Mitochondria, M. X. Mitochondrial bodies formed by active proliferation, N. Nucleolus, N. Nucleolar granules of the first phase of extrusion, N. X. Nucleolar grains proliferating in the cytoplasm, N. Y. Nucleolar yolk spheres.

Plate 1, fig. 1. × 1400. Young oocytes from the germinal epithelium, showing Golgi apparatus in juxta-nuclear position (upper cell), and beginning to fragment (lower cells).

Plate 1, fig. 2. × 650. Older oocyte, showing scattered apparatus.

Plate 1, fig. 3. × 1400. Part of still older oocyte, Golgi bodies breaking into small grains.

Plate 1, fig. 4. × 1400. Young oocyte, showing later stage of first nucleolar extrusion. Possibly abnormal.

Plate 1, fig. 6. × 650. Oocyte which has left germinal epithelium, showing mitochondria collected to a cloud, and nucleolus broken up.

Plate 2, fig. 7. × 650. Older oocyte, showing round mitochondrial body; nucleolar fragments proliferating both in nucleus and cytoplasm.

Plate 1, fig. 8. × 650. Oocyte, showing mitochondrial body like that of fig. 7; other mitochondria in scattered clouds.

Plate 1, fig. 9. × 650. Older oocyte, showing scattering of mitochondria beginning; nucleolus broken up to small fragments.

Plate 2, fig. 10. × 650. Part of oocyte, showing mitochondria scattered in strings through the cytoplasm; nucleolus finely divided, some of its fragments passed out to the cytoplasm.

Plate 2, fig. 11. Part of oocyte, showing mitochondria evenly distributed through the cytoplasm. Nucleolar yolk beginning to form.

Plate 2, fig. 12. × 350. Part of large oocyte laden with nucleolar yolk spheres in all stages of development.

Plate 2, fig. 13. × 1400. Cell from follicle, showing dark granules in cytoplasm.

Plate 2, fig. 14. × 1400. Typical follicle cell, showing clear cytoplasm, and nucleus with few nucleolar grains.

Figs. 1–3 are drawn from material fixed by Da Fano's method, and mounted without staining; figs. 4–14 are from Flemming-without-acetic preparations, stained in iron hæmatoxylin.

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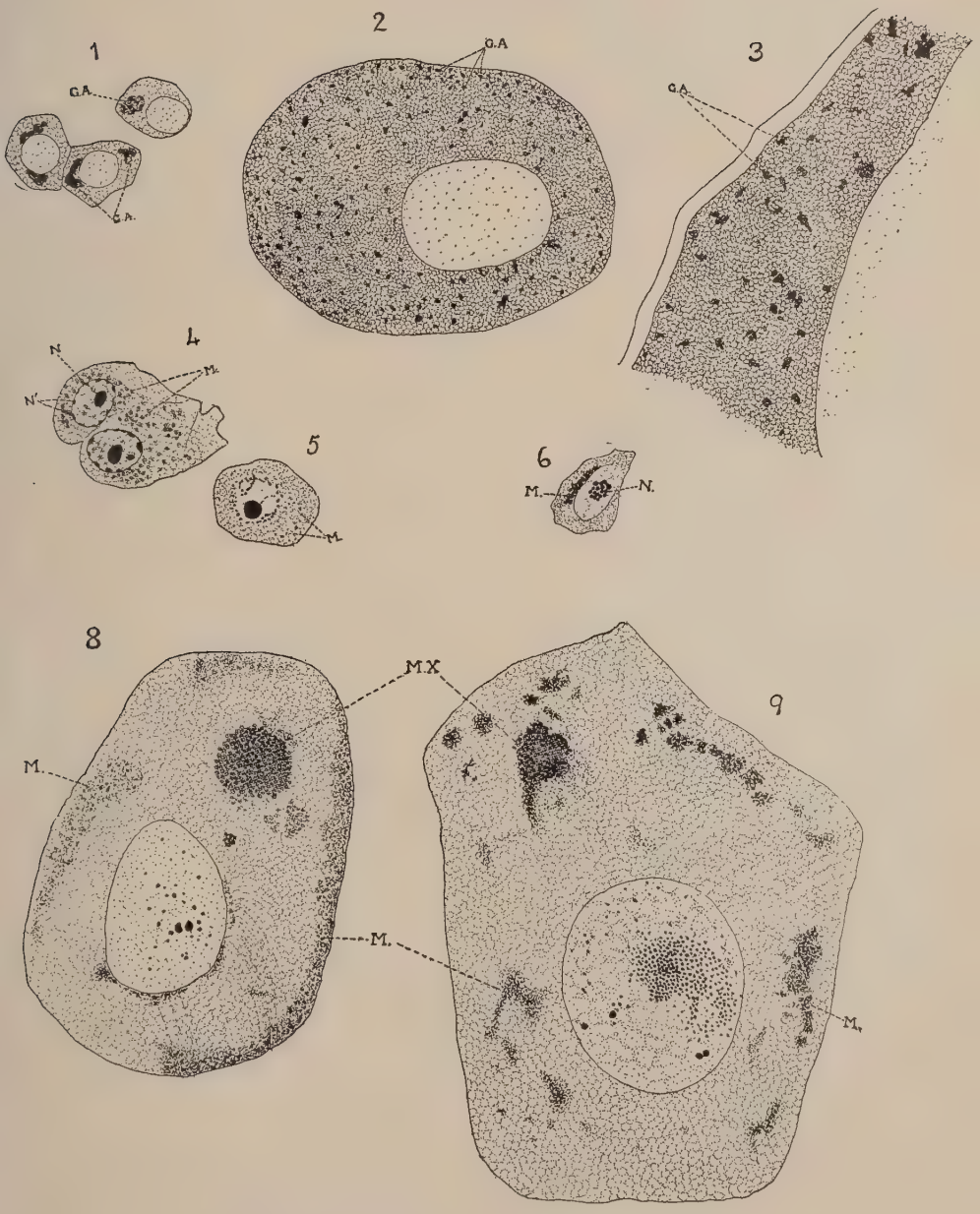
Note.—In a recent letter to "Nature" (August 2nd, 1924), Vishwa Nath has criticized some of the conclusions arrived at in this paper. He denies the fact that the nucleolus breaks up in the young oocyte, and states that it persists throughout oogenesis, budding off the fragments which are extruded into the cytoplasm. If this be so, it is difficult to explain the appearance shown in the nucleus in fig. 6, a stage which is common in my preparations. On his view we would have to assume that one of the fragments shown is the nucleolus, and that the others, which are approximately equal to this in size, are the buds. This seems an unnecessarily strained interpretation, especially in view of the complete absence of stages intermediate between Figs. 4 and 6 from my preparations. Serial sections of many young and half-grown oocytes have been examined, and, except in rare and doubtfully normal cases, nothing which could be interpreted as a single nucleolus has been found, and all the larger nucleolar particles seem to bud indiscriminately. The large oxyphil nucleolus described by Vishwa Nath in the full-grown oocyte must be derived from one or more of these basophil particles, a process already described in certain mollusca.

Owing to difficulty in obtaining full-grown oocytes, this point has not been investigated in the present paper.

The fact that yolk is formed by nucleolar extrusion is supported by Hogben's work on insect oogenesis, and I have no hesitation in stating it to be true for Lithobius also.

With regard to Vishwa Nath's account of the "fatty degeneration" of the Golgi bodies to form yolk, I have no evidence to offer either in support of, or against, his observations. It is doubtful, however, if the term "fatty degeneration" should be used to describe the loading up of the Golgi bodies with various fats.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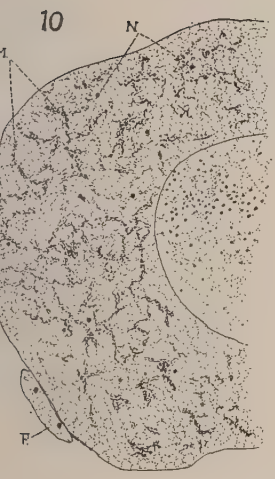

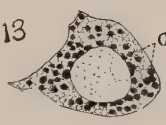

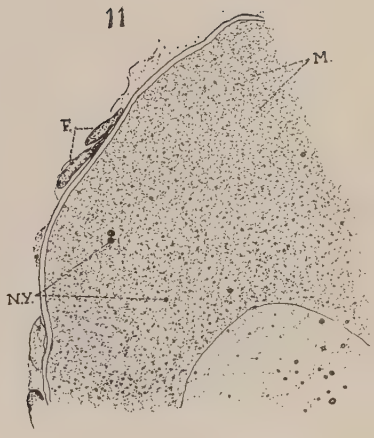

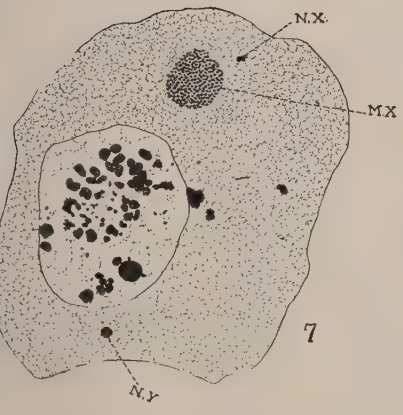

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No. 4.

THE DETERMINATION OF THE MOST ECONOMIC SIZE OF PIPE-LINE FOR WATER-POWER INSTALLATIONS.

By H. H. JEFFCOTT.

[Read October 28. Printed November 28, 1924.]

1. The lay-out of a scheme for the development of hydro-electric power usually involves the provision of a pipe-line to convey the water from the reservoir or head-race to the turbines. The choice of the size of the pipes, to secure the most economical results when performing a specified duty, depends mainly on effecting an advantageous compromise between two opposing conditions. On the one hand the weight of material required in the pipe increases with increase of size; while on the other hand diminution in size of the pipe, with consequent increase in velocity of the water, leads to increased loss of head due to friction in the pipe, and, therefore, of power at the turbines.

Thus it will be seen that two important factors in the determination of the best size are the values of power and of the material of the pipe at the position in question. The choice of size is to be determined by securing that the sum of all the charges in connexion with the pipe-line (including the value of the power lost in the pipe), when reckoned over a long period of years, shall be a minimum. We will proceed to examine these charges in detail, and obtain algebraic expressions for them.

2. The following notation will be employed:—

Let Q cubic feet be the quantity of water to be conveyed per second at a particular time by n circular pipes, each of internal diameter D feet at a position l feet, measured along the pipe, distant from the reservoir.

Let the thickness of each pipe be t inches at the position l along its length, and let this position be at a level h feet vertically below the water-level of the reservoir or head-race, which is supposed to remain practically constant.

Let the minimum thickness that it is desirable to adopt for the pipes from practical considerations be t' inches.

Let the length of the low-pressure pipe, for which the thickness is t' throughout, be l_1. Further, let the high-pressure pipe, for which the thickness at any point is t, extend from the position l_1 to a position distant l_2 from the reservoir. Suppose that thereafter from l_2 to the lower end the pipe-line consists of n tunnels.

Let the whole length of the pipe-line from reservoir to turbines be L feet and the total head be H feet. Let the slope of the pipe-line be such that the head h is related to the length l by the equation $h = \phi(l)$.

Let S be the safe working tensile stress in lbs. per sq. inch of the material of which the pipe is composed multiplied by the efficiency of the joint. Thus the value of S for steel pipes may be taken as 8,000 lbs. per sq. inch.

Let w be the weight in pounds per cubic foot of the pipe material.

Let V feet per second be the average velocity of flow of water in the pipes corresponding to a total discharge of Q cubic feet per second, and let it be assumed that the pipes, if more than one in number, shall simultaneously operate at equal average velocities, and that these velocities are steady and free from surging oscillation.

Let the loss of head per unit of length (foot) in each of the pipes due to pipe friction, to effects of bends and changes of section (if any), and to loss at entrance be represented with sufficient exactness by $\frac{\mu V^2}{D}$, where μ is a coefficient depending on the average condition of roughness of the pipes, and on the bends, changes of section, etc., in the pipe-line.

Let q be the value in pounds per ton of the pipe material delivered at the site and erected.

Let aD^2 pounds sterling be that part of the cost of construction of tunnels per foot run that depends on the square of the diameter, i.e. excavation; and let bD pounds sterling be that part of the cost per foot run that depends on the first power of the diameter, i.e. facing of the inside of the tunnel.

Let p be the value in pence per K.W.hr. of power as delivered to the load mains.

With plant operating at an industrial load factor the generators, transformers, switch gear, and transmission line are provided of a capacity capable of carrying the peak load, and they would, therefore, also be capable of carrying the power lost in the pipe-lines were it available. Accordingly, as the plant already installed is capable of generating the extra power lost in the pipe-lines were it rendered partly or wholly available, the value of such lost power is to be reckoned at the value corresponding to its delivery as generated. Hence the value of p is that of the power as delivered to the distribution mains. On the other hand, with an ideal load factor of unity the value of the power lost in the pipe-lines should be reckoned at its value as supplied to the turbines, for in this case extra plant would be required to generate the corresponding electric power and the cost of generation would be appreciable. But this ideal case may be omitted here.

Let η be the combined efficiency, for the load considered, of generation, transformation, and transmission of electrical power to the point of delivery to the mains, reckoned from the water-power supplied to the turbines.

Let m be the cost in pounds sterling per water horse-power of turbines or of turbines and generators as the case may be, and let m' be the value in pounds sterling per water horse-power of that part of the power-house that may be regarded as utilised for housing the turbines or the turbines and generators as the case may be.

Let r be the rate of interest on money for the period in question. Also let i be the rate for depreciation and sinking fund on the pipe-line, so that, at the end of its useful life, this rate of interest on the capital out-lay on the pipe-line will have covered all the repairs required on it, and will in addition provide a fund capable of paying off the initial capital out-lay less the scrap value of the pipes in situ at the end of the period. Let i' be the corresponding figure for tunnels.

In like manner let j be the rate of similar depreciation and sinking fund on turbines or on turbines and generators as the case may be, and j' on the power-house, allowing for repairs and scrap value in each case.

3. The necessary thickness of the wall of the pipe is obtained from consideration of the stresses in a longitudinal seam of the pipe of length say 1 inch.

Consider the stresses in such ring element at two diametrically opposite sections. On one half of the ring the water exerts a pressure P lbs. per sq. inch which gives a resultant force perpendicular to the diametral section of amount 12 PD lbs. This force is supported by the stress in the material of the pipe at the section considered, which gives a force approximately equal to 2 tS lbs., the thickness of the pipe being supposed small in comparison with its diameter. Hence

$$t = \frac{6PD}{S} = 2{\cdot}6\frac{hD}{S}$$

where S is the tensile stress in the material.

It is to be noted that the stress due to the effect of water pressure on a circumferential section of the pipe (i.e. perpendicular to its length) is less than that in the longitudinal seam, so that the thickness of pipe required to support the stress in a longitudinal seam is more than sufficient to support the stress in a circumferential seam.

While the thickness of the pipe is to be determined by the formula just obtained, it will be found to give an impracticably small thickness when the head is very low. It is, therefore, necessary to decide upon a practical limit to the thickness, having regard to ability to withstand accidental damage, corrosion, etc. Let this minimum thickness be t'. The value of t' depends on the material of the pipe, and for steel and iron may be taken as $\frac{1}{4}$ inch. The thickness t' is adopted for that portion of the pipe for which this value is greater than $2{\cdot}6\frac{hD}{S}$.

Thus, in general, the thickness of the pipe wall is $t = 2{\cdot}6\frac{hD}{S}$ or t', whichever be the greater.

4. The weight in tons of material in the n pipes, of constant thickness t' from 0 to l_1, and of varying thickness t from l_1 to l_2, is

$$\frac{\pi nwt}{26880}\int_0^{l_1} Ddl + \frac{\pi nw}{26880}\int_{l_1}^{l_2} tDdl.$$

The cost of the pipe-line delivered and erected on the site may be regarded with sufficient exactness as being proportional to the weight of material of which it is composed.

Hence the value in pounds sterling of the n pipe-lines erected is q times the weight in tons given above. The value of q is to be chosen to correspond to the sizes of pipe likely to be adopted, and further it is assumed to remain constant for a limited range of sizes above and below the most economic. If this assumption is only approximately true the result obtained for the most economic size will likewise be only approximate, but a more precise result can then be obtained, if desired, by working out the total annual costs for a number of sizes of pipe in the neighbourhood of the approximate size obtained by the method here given, and choosing the size for which the total annual cost is least.

The total average annual charge in pounds per annum for the material

over the whole life of the pipe-line is, for the n pipe-lines, of total length l_2 feet,

$$\frac{\pi nwq\,(r+i)t'}{26880}\int_0^{l_1} Ddl + \frac{\pi nwq\,(r+i)}{26880}\int_{l_1}^{l_2} tDdl.$$

Since

$$t = 2{\cdot}6\,\frac{hD}{S} = 2{\cdot}6\,\frac{\phi(l)\,D}{S},$$

we find on substitution that the annual charge in pounds sterling for the pipe-line material is

$$\frac{nwq\,(r+i)\,t'}{8552{\cdot}7}\int_0^{l_1} Ddl + \frac{nwq\,(r+i)}{3289{\cdot}5\,S}\int_{l_1}^{l_2}\phi(l)\,D^2dl.$$

5. Part of the pipe-line may sometimes consist of tunnels through rock.

For this the cost depends on the amount and nature of the excavation required, and on the work involved in lining or facing the inside surface. For ordinary cases we may then take the cost per unit length (foot) of the tunnel to be the sum of two terms, one being proportional to the area of section, and the other to the length of its circumference.

Thus if the n tunnels extend from position l_2 to L, the cost is

$$na\int_{l_2}^{L} D^2dl + nb\int_{l_2}^{L} Ddl.$$

where a and b depend on the charges for excavation and for lining the tunnel respectively.

The annual charge in pounds sterling is—

$$na\,(r+i')\int_{l_2}^{L} D^2dl + nb\,(r+i')\int_{l_2}^{L} Ddl.$$

6. We have next to estimate the value of the power lost in the pipes. For one pipe of diameter D at position l, where the average velocity of discharge is V feet per second, the loss of head in the short length dl is approximately

$$\mu\frac{V^2dl}{D}.$$

The discharge through the pipe is $\frac{\pi}{4}D^2V$ cubic feet per second. As the water horse-power corresponding to a head H feet and discharge Q cubic feet per second is $\frac{HQ}{8{\cdot}83}$, we see that the water horse-power lost in the length dl of the pipe is

$$\frac{\pi\mu}{35{\cdot}32}DV^3dl.$$

Hence the loss of power in the n pipes is

$$\frac{\pi n\mu}{35{\cdot}32}\int_0^{L} DV^3dl.$$

Since

$$\frac{\pi}{4} D^2 V = \frac{Q}{n},$$

the loss of power in the n pipes is

$$\frac{\mu Q^3}{5{\cdot}45 n^2} \int_0^L \frac{dl}{D^5} \text{ horse-power.}$$

It thus appears that at any time when the discharge through the pipes is Q cubic feet per second the loss of power is proportional to Q^3.

The value in pounds sterling of this power during a year for which the cube root of mean cube of the discharge is Q_m is

$$\frac{5p\eta\mu Q^3{}_m}{n^2} \int_0^L \frac{dl}{D^5}.$$

7. Now in general the output generated at a power station varies greatly from time to time throughout a typical year, sometimes being down almost to zero, and at times of peak load being in the neighbourhood of the full plant capacity of the station.

In estimating the value of the power lost during such a year it is necessary to take for Q a value equal to the cube root of the arithmetic mean of the cubes of Q. This, of course, is not the same as the arithmetic mean of Q throughout the year, and in general it is greater than the latter.

As an example, if the discharges or corresponding loads during the year, when plotted to a time base in order of increasing magnitude, show a straight line variation from 0 to Q_1, it may be seen that while the arithmetic mean value is 0·5 Q_1, the cube root of mean cube of Q is 0·63 Q_1, so that the ratio of the latter to the former is 1·26.

Further, if the plotted discharges during the year exhibit variation along a straight line from the lowest value Q_0 to the highest value Q_1, the arithmetic mean value is 0·5 $(Q_0 + Q_1)$, whereas the cube root of mean cube of Q is

$$0{\cdot}63 \sqrt[3]{(Q_0 + Q_1)(Q_0^2 + Q_1^2)},$$

and the ratio of the latter to the former is

$$1{\cdot}26 \sqrt[3]{\frac{Q_0^2 + Q_1^2}{(Q_0 + Q_1)^2}}.$$

Or, again, as a further example, if the plotted discharges or loads during the year vary along a complete sine curve of amplitude b and mean height a, so that the minimum value is $Q_0 = a - b$ and the maximum value is $Q_1 = a + b$, then the arithmetic mean value is $a = \frac{1}{2}(Q_0 + Q_1)$, while the cube root of mean cube of Q is

$$\sqrt[3]{a\left(a^2 + \frac{3}{2}b^2\right)} \quad \text{or} \quad \tfrac{1}{2}\sqrt[3]{(Q_0 + Q_1)\left\{\frac{5}{2}(Q_0^2 + Q_1^2) - Q_0 Q_1\right\}},$$

and the ratio of the latter to the former value is

$$\sqrt[3]{1 + \frac{3b^2}{2a^2}} \quad \text{or} \quad \sqrt[3]{1 + \frac{3}{2}\left(\frac{Q_1 - Q_0}{Q_1 + Q_0}\right)^2}.$$

In general the determination of the cube root of mean cube of the discharges during the year is to be effected by plotting as ordinates the values of the discharge at the different hours of the year. These equidistant ordinates must then severally be cubed and the cubes be plotted as a new series of ordinates. The mean cube is got by measuring the area of this new curve above the time base and dividing the result by the length of the time base. The cube root of the resultant quantity is the figure required, and from it the value of the power loss in the pipes for the year may be determined. The cube root of the mean of the cubes of the discharge divided by the arithmetical mean of the discharge during the period considered may conveniently be called the hydraulic form factor of the load, while the square root of mean square of the electric current divided by the arithmetical mean of the current during the period considered may in like manner be called the electrical form factor of the load, and has a corresponding application in the determination of the most economic size of electric transmission line. (See Sci. Proc. R.D.S., vol. xvii, pp. 74, 75.)

8. It is desirable now to consider the influence of the loss of head in the pipe-line on the plant capacity of the power station.

Ordinarily the power station will be designed to be capable not merely of giving a certain average output of power throughout the year, but also of supplying a peak load, for which a sufficient plant capacity must be installed.

It will be clear that in providing for such peak load the effect of loss of head in the pipes becomes of serious importance. For with the great discharge necessary to meet the peak requirements the loss of head in the pipe-line (which varies approximately as the square of the discharge) becomes very large, so that the discharge must be still further increased to secure the desired peak power.

The effect of this on the plant is twofold. The turbines will ordinarily be designed to operate most efficiently on a head in the neighbourhood of that available when the total discharge is low, or at all events not far removed from mean conditions. Accordingly, at peak load the generating sets are operating at considerably under the designed head, and the efficiency is impaired, especially if the peak conditions are severe and the pipe-line long and of restricted diameter. Further, as the full output of the turbines is decreased approximately in proportion to the decrease of nominal head under peak conditions, while the discharge capacity of the turbines is determined by their size, it is clear that either an increase in the plant installed will be necessary to supply the peak output of the station, or turbines of larger discharge capacity must be adopted, if the pipe-line is unduly restricted. Accordingly, when comparing the relative advantages of two sizes of pipe line it is necessary to take into consideration the increased plant (or turbine) capacity (at rated head) required with the smaller size of pipe-line to supply the peak load for which the station is being designed. Whether turbines of greater discharge capacity are to be provided, or additional turbine and generator sets are to be installed, depends on the relative disadvantage of the impaired efficiency at part gate in the one case, or the extra cost of the generators in the other.

In any particular case if Q_1 be the maximum discharge required of the pipe-lines at peak load, then the loss of power in the n pipes is (see sect. 6)—

$$\frac{\mu Q_1^3}{5{\cdot}45n^2}\int_0^L \frac{dl}{D^5} \text{ horse-power.}$$

Hence increased plant (or turbines) to convert water horse-power of this amount must be provided over and above that required to meet the same peak

output on the assumption that pipe friction did not exist. Thus the friction loss of head in the pipe-lines increases the size of the turbines or the amount of plant otherwise necessary to meet the peak output, for by causing a reduction in the effective head it requires a corresponding increase in the discharge, and the turbine capacity must be ample to pass this increased discharge.

The increase of capacity of the plant (turbines and generators or turbines alone as the case may be) thus involved is

$$\frac{\mu Q_1^3}{5{\cdot}45n^2}\int_0^L \frac{dl}{D^5} \text{ water horse-power,}$$

and the value in pounds sterling per annum of the interest and depreciation on this additional plant is

$$\frac{m(r+j)\mu Q_1^3}{5{\cdot}45n^2}\int_0^L \frac{dl}{D^5}.$$

Further, in addition to provision of extra turbines or turbines and generators, there will be involved an increase in the size of the power-house in respect of the additional plant.

The value in pounds sterling per annum of the interest and depreciation on this additional part of the power-house is

$$\frac{m'(r+j')\mu Q_1^3}{5{\cdot}45n^2}\int_0^L \frac{dl}{D^5}.$$

Both j and j' are to be chosen so as to include the extra annual charges (if any) for the operation of the increased plant and power-house.

9. If the load on the power-house were an unvarying one throughout the year, then with a restricted pipe-line the average power output would be less than with a large pipe-line owing to the greater friction loss in the pipes. Accordingly a smaller average power is available for generation at a uniform rate throughout the year, and a smaller horse-power of plant is therefore needed.

This may not affect the size of the turbines seriously, for the discharge is a fixed quantity, while the head depends on the pipe-line friction. Under such circumstances we may suppose that the turbine size is the same whether the pipe friction be large or small.

In general, however, the load factor will not be one hundred per cent., and the output from the power-station will be a varying one. Adequate plant capacity must be provided to supply the peak load.

10. We can now collect together the various annual charges, and the problem to be solved consists in choosing D so that the sum of all the annual charges is a minimum.

The materials of the pipe-lines and construction of the tunnels lead to an annual charge of

$$\frac{nwq(r+i)t'}{8552{\cdot}7}\int_0^{l_1} Ddl + \frac{nwq(r+i)}{3289{\cdot}5\ S}\int_{l_1}^{l_2} \phi(l)D^2dl$$
$$+ na(r+i')\int_{l_2}^{L} D^2dl + nb(r+i')\int_{l_2}^{L} Ddl.$$

The loss of power gives an annual charge of

$$\frac{5p\eta\mu Q_m{}^3}{n^2}\int_0^L \frac{dl}{D^5}.$$

The increased plant (turbines or turbines and generators) required for the supply of a specified peak load owing to the friction loss of head gives rise to an annual charge of

$$\frac{m(r+j)\mu Q_1{}^3}{5{\cdot}45\,n^2}\int_0^L \frac{dl}{D^5}.$$

The corresponding increase in size of the power-house leads to an annual charge of

$$\frac{m'(r+j')\mu Q_1{}^3}{5{\cdot}45\,n^2}\int_0^L \frac{dl}{D^5},$$

where Q_1 is the maximum value of the total discharge through the n pipe-lines.

For convenience write :—

$$A = \frac{nwq\,(r+i)\,t'}{8552{\cdot}7}, \qquad B = \frac{nwq\,(r+i)}{3289{\cdot}5\,S}, \qquad C = na\,(r+i'),$$

$$E = nb\,(r+i'), \qquad F = \frac{5p\eta\mu Q_m{}^3}{n^2} + \frac{m\,(r+j)\,\mu Q_1{}^3}{5{\cdot}45\,n^2} + \frac{m'\,(r+j')\,\mu Q_1{}^3}{5{\cdot}45\,n^2}.$$

We have therefore to find the values of D for which

$$A\int_0^{l_1} Ddl + B\int_{l_1}^{l_2}\phi\,(l)\,D^2dl + C\int_{l_2}^{L} D^2dl + E\int_{l_2}^{L} Ddl + F\int_0^L \frac{dl}{D^5}$$

is a minimum.

Or again,

$$\int_0^{l_1}\left(AD + \frac{F}{D^5}\right)dl + \int_{l_1}^{l_2}\left(B\phi\,(l)\,D^2 + \frac{F}{D^5}\right)dl + \int_{l_2}^{L}\left(CD^2 + ED + \frac{F}{D^5}\right)dl$$

is a minimum.

11. The determination of the law connecting D and l so as to make the sum of these definite integrals a minimum requires the application of the calculus of variations.

The limits l_2 and L are fixed, and, accordingly, the law of D for the tunnel section must be chosen so as to make the definite integral between those limits a minimum.

Hence

$$\delta\int_{l_2}^{L}\left(CD^2 + ED + \frac{F}{D^5}\right)dl = 0$$

where δ is the variation of the quantity on which it operates.

It follows that

$$\int_{l_2}^{L}\left(2CD + E - \frac{5F}{D^6}\right)\delta D dl = 0,$$

and as δD, though always small, is perfectly arbitrary, this requires

$$2CD + E - \frac{5F}{D^6} = 0.$$

This equation shows that, for the tunnel, D is independent of l, and the value of D is obtained from its solution.

For the pipe-line proper we require

$$\int_0^{l_1}\left(AD + \frac{F}{D^5}\right)dl + \int_{l_1}^{l_2}\left(B\phi(l)D^2 + \frac{F}{D^5}\right)dl$$

to be a minimum. Here l_2 is fixed, but the value of l_1 must be determined to satisfy the conditions of the problem.

First suppose that the value of l_1 has been ascertained, and that it is fixed and known. Then the sum of the two definite integrals is a minimum when the law of D is so chosen for each integral as to make it a minimum.

The first of the integrals is a minimum when

$$\delta\int_0^{l_1}\left(AD + \frac{F}{D^5}\right)dl = 0 \quad \text{or} \quad \int_0^{l_1}\left(A - \frac{5F}{D^6}\right)\delta D dl = 0,$$

and as δD is arbitrary, it follows that $D^6 = \dfrac{5F}{A}$. Thus from 0 to l_1 the value of the diameter is constant (= D' say). The minimum value of the integral is

$$\int_0^{l_1}\left(AD + \frac{F}{D^5}\right)dl = \frac{6Fl_1}{D^5}.$$

The second of the integrals is a minimum when

$$\delta\int_{l_1}^{l_2}\left(B\phi(l)D^2 + \frac{F}{D^5}\right)dl = 0 \quad \text{or} \quad \int_{l_1}^{l_2}\left(2B\phi(l)D - \frac{5F}{D^6}\right)\delta D dl = 0,$$

which leads to $D^7 = \dfrac{5F}{2B\phi(l)}$.

Thus, from l_1 to l_2 the value of the diameter is a function of the position l.

The minimum value of the integral is therefore

$$\int_{l_1}^{l_2}\left(B\phi(l)D^2 + \frac{F}{D^5}\right)dl = \int_{l_1}^{l_2}\frac{7F}{2D^5}dl.$$

We have now to choose l_1 so that the sum of these two smallest values of the two definite integrals in question shall be a minimum, subject, however, to the conditions that from 0 to l_1 $\dfrac{2{\cdot}6\phi(l)D}{S}$ is not to be greater than the specified value t', while from l_1 to l_2 the value of t is never to be less than t'.

Omitting for the moment these conditions, we proceed to find by the ordinary method of the calculus the value of l_1 for which

$$\frac{6F}{D^5} l_1 + \frac{7F}{2} \int_{l_1}^{l_2} \frac{dl}{D^5} \text{ is a minimum.}$$

Substituting the respective values of D in the two parts of this expression, it becomes

$$6F^{1/6}\left(\frac{A}{5}\right)^{5/6} l_1 + \frac{7}{2} F^{2/7}\left(\frac{2B}{5}\right)^{5/7} \int_{l_1}^{l^2} \left[\phi(l)\right]^{5/7} dl.$$

Differentiating this expression with respect to l_1 and equating to zero we find

$$6F^{1/6}\left(\frac{A}{5}\right)^{5/6} - \frac{7}{2} F^{2/7}\left(\frac{2B}{5}\right)^{5/7}\left[\phi(l_1)\right]^{5/7} = 0$$

or $$\phi(l_1) = \left(\frac{6}{7}\right)^{7/5} \times 2^{2/5} \times \left(\frac{1}{5}\right)^{1/6} \times \frac{A^{7/6}}{BF^{1/6}} = 0{\cdot}81319 \frac{A^{7/6}}{BF^{1/6}} = 0{\cdot}26672 \left(\frac{BS7t'7}{F}\right)^{1/6}$$

This gives l_1 in terms of the known constants A, B, and F.

We have now to see if the conditions of the problem are satisfied by this solution for l_1.

We have

$$\phi(l_1) = 0{\cdot}81319 \cdot \frac{A^{7/6}}{BF^{1/6}}; \quad D' = \left(\frac{5F}{A}\right)^{1/6}; \quad t' = \frac{2{\cdot}6A}{BS}.$$

Hence $$\frac{2{\cdot}6\ \phi(l_1) D'}{S} = 1{\cdot}0634 . t'.$$

Likewise, since

$$D_1 = \left(\frac{5F}{2B\phi(l_1)}\right)^{1/7} \qquad t_1 = \frac{2{\cdot}6\,\phi(l_1) D_1}{S} = 0{\cdot}95471\, t'.$$

Further, $D_1 = 0{\cdot}8978\ D'$.

Thus, the value of l_1 that makes the sum of the definite integrals a minimum complies with sufficient accuracy for practical requirements with the specified conditions of the problem in regard to the value of the thickness of the pipe.

Note that the position l' on the low pressure part of the pipe for which

$$\frac{2{\cdot}6\phi(l')D'}{S} = t' \quad \text{is given by} \quad \phi(l') = 0{\cdot}76472 \frac{A^{7/6}}{BF^{1/6}}.$$

Also the position l'' on the high pressure part for which

$$t = \frac{2{\cdot}6\phi(l'')D}{S} = t' \quad \text{is determined by} \quad \phi(l'') = 0{\cdot}85837 \frac{A^{7/6}}{BF^{1/6}}.$$

These two positions l' and l'' lie on either side and close to the position l_1, and between these positions the pipe-line should have a gradual change of diameter from that required in the low pressure part to the requisite diameter in the high pressure part.

12. The results of the last paragraph may be summarized for reference. The fall of the pipe-line below the horizontal is represented by $h = \phi(l)$. The low

pressure part of the pipe-lines extends from 0 to l_1, and the high pressure part from l_1 to l_2. We have supposed the tunnels to extend from l_2 to L.

Then $$\phi(l_1) = 0{\cdot}8132\frac{A^{7/6}}{BF^{1/6}}$$ which determines l_1.

From 0 to l_1, $$D = 1{\cdot}3077\left(\frac{F}{A}\right)^{1/6};\quad t = t'.$$

From l_1 to l_2, $$D = 1{\cdot}1399\left(\frac{F}{B\phi(l)}\right)^{1/7};\quad t = \frac{2{\cdot}6\phi(l)\,D}{S}.$$

From l_2 to L, D satisfies the equation $2CD^7 + ED^6 - 5F = 0$ where

$$A = \frac{nwq\,(r+i)\,t'}{8553};\qquad B = \frac{nwq\,(r+i)}{3290\,S};$$

$$C = na\,(r+i');\qquad E = nb\,(r+i');$$

$$n^2F = 5p\eta\mu Q_m^3 + 0{\cdot}1835\mu Q_1^3\{m\,(r+j) + m'\,(r+j')\}.$$

13. In most cases the relation between the head (or depth below reservoir level) and the length along the pipe $h = \phi(l)$ cannot be represented by a simple mathematical law.

This is immaterial if we are given the curve of slope of the pipe-line. For we have only to determine the value of $0{\cdot}8132\ \dfrac{A^{7/6}}{BF^{1/6}}$, which is equal to $\phi(l_1)$ or h_1, and mark the point on the curve corresponding to this value of the head. Thus, l_1 is determined. Moreover, in the equation for D between l_1 and l_2, we write for $\phi(l)$ the value of h got from the given curve of slope corresponding to the position l for which the value of D is being determined.

When we know the value of D for each part of the pipe-line the total of the annual charges can be evaluated from the sum of the definite integrals given at the end of paragraph 10.

14. The foregoing calculations have been made for an arrangement having n pipe-lines followed by n tunnels. But it is obvious that the reverse arrangement or any other mixed arrangement of tunnels and pipe-lines can equally be dealt with by the preceding theory, since the laws of diameter for the tunnel and for the pipe-line are independent of one another.

15. As an example, consider the case of a steel pipe-line 5000 feet long followed by tunnel 1000 feet long, all sloping uniformly downwards through a total fall of 200 feet from the water level in the reservoir.

Maximum discharge $Q_1 = 1000$ c.f.s.

Cube root of mean cube of discharge is $Q_m = 350$ c.f.s.

The number of pipe-lines and of tunnels is to be two, the discharge being supposed to be always equally divided between them.

Let the pipes be of steel, for which

$S = 8000$ lbs. per sq. inch, and $w = 480$ lbs. per cubic foot.

Let $q = £30$ per ton, and $p = 1$d. per unit.

For illustrative purposes take

$r = {\cdot}06$, $i = {\cdot}06$, $i' = {\cdot}04$, $j = {\cdot}07$, $j' = {\cdot}05$,

$m = 3$, $m' = 1{\cdot}5$, $a = 0{\cdot}1$, $b = 0{\cdot}1$, $\eta = 0{\cdot}7$,

$\mu = {\cdot}0004$, $t' = 0{\cdot}25$ inch.

Hence

$$A = 0{\cdot}101, \quad B = 0{\cdot}0001312, \quad C = 0{\cdot}02, \quad E = 0{\cdot}02, \quad F = 25200,$$

$$\phi(l) = \frac{1}{30}l, \qquad \frac{1}{30}l_1 = 0{\cdot}8132 \times \frac{{\cdot}069}{{\cdot}0001312 \times 5{\cdot}415} \qquad \therefore\ l_1 = 2370 \text{ feet.}$$

From 0 to l_1, $D = 1{\cdot}3077 \times \dfrac{5{\cdot}415}{{\cdot}682} = 10{\cdot}382$ feet.

From l_1 to l_2 $D = 1{\cdot}1399 \times 15{\cdot}253 \times (30)^{1/7} \times \dfrac{1}{l^{1/7}} = \dfrac{28{\cdot}264}{l^{1/7}}$;

$$\therefore\ D_1 = \frac{28{\cdot}264}{3{\cdot}018} = 9{\cdot}365 \text{ feet}; \qquad D_2 = \frac{28{\cdot}264}{3{\cdot}376} = 8{\cdot}372 \text{ feet.}$$

The thickness of the pipe from 0 to l_1 is 0·25 inch, and at l_2 is 0·45 inch.

From l_2 to L, D satisfies

$$0{\cdot}04\,D^7 + 0{\cdot}02\,D^6 - 126000 = 0 \quad \text{or} \quad 2D^7 + D^6 - 6{\cdot}3 \times 10^6 = 0.$$

This gives 8·4 feet as a value of D.

Hence, the most economic pipe-line under the conditions hypothesized consists of two steel pipes each of 10′ 4″ internal diameter from the reservoir to a point 2370 feet therefrom. Thereafter the best pipe diameter changes gradually but rapidly to 9′ 4″ diameter, and gets smaller gradually to 8′ 4″ at 5000 feet. There the tunnels commence gradually but rapidly enlarging from the pipe-lines to a diameter of 8′ 5″, which size is maintained to the end of the system at 6000 feet from the reservoir.

In practice, of course, the sizes should be arranged in lengths of suitable standard diameter and thickness, so as to approximate to the theoretically best solution, and the changes of section of the pipe should be effected gradually to obviate shock and consequent loss of energy to the water.

No. 5.

THE INTERPRETATION OF CERTAIN EMPIRICAL STANDARDS IN THEIR APPLICATION TO IRISH BUTTER.

By GEO. BROWNLEE, B.Sc., F.I.C.,

of the State Laboratory, Dublin, and formerly attached to the Chemical Division of the Department of Agriculture and Technical Instruction for Ireland.

[COMMUNICATED BY PROFESSOR RYAN.]

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It has been known for some years that genuine Irish butter may, at certain seasons, give figures on analysis which do not conform with the generally recognised standards of purity. This particularly applies to the Reichert-Wollny test, i.e. the test on which analysts chiefly rely in arriving at a decision as to the genuineness of butter.

As long ago as 1902 Mr. A. Poole Wilson, Chief Inspector in Dairying of the Department of Agriculture and Technical Instruction, in giving evidence[1] before the Departmental Committee on Butter Regulations quoted from his own experience instances in which butters gave abnormally low Reichert-Wollny numbers. In spite of this and other evidence, the Committee in their Final Report recommended that in the Reichert-Wollny test the number 24 should be officially adopted as the lower limit, and that any butter giving a number below 24 should be assumed to be adulterated. In order to emphasize the unreliability of the 24 limit at certain seasons of the year, and to prevent the unfair prosecution of Irish butter merchants, the Department of Agriculture and Technical Instruction initiated an investigation extending over a period of thirteen months in the years 1908–9. The results of this investigation, carried out by the writer, were published in the Department's Journal, vol. x, No. 3, under the title "Irish Butter and the Reichert-Wollny Standard," and showed that out of 300 samples 16·2 per cent. gave a Reichert-Wollny number below 24, the lowest being 20·1. The low results were all obtained during the months of November, December, and January.

As cases have again occurred in recent winters, in which the purity of consignments of Irish Butter has been questioned on account of its having given low Reichert-Wollny figures, the Department decided that it would be advisable to conduct further investigations. At the same time opportunity was taken to extend the investigation by having a number of additional tests made on the samples, with the object of discovering, if possible, whether any

[1] C.D. 1039 and 1750.

of these tests might prove to be more reliable than the Reichert-Wollny test. The Department's Creamery Section selected eleven creameries in the south-west of Ireland as suitable for the object in view, and organised the collection of the samples therefrom by their inspectors, so that the genuineness of the samples can be vouched for. It should be noted that the butters represent the product of the mixed milk from various herds. The investigation commenced early in October, 1923, and continued till early March, 1924, thus covering the period during which butters giving abnormally low Reichert-Wollny numbers are generally produced. The inspectors took 112 samples of butter or of the cream from which butter was to be made, and despatched these to the Department's Chemical Advisory Division for analysis. In addition 15 samples were submitted from the Department's Agricultural School, Clonakilty. Omitting consideration of the Clonakilty results for the present, as the conditions obtaining there are not those of an ordinary commercial creamery, the following results were obtained:—

Reichert-Wollny Number.—In this test glycerol-soda solution was used for the saponification of the fats, and the standard Polenske apparatus for the distillation of the volatile fatty acids, the heating arrangements being modified to ensure that the distillation could be easily accomplished in the specified time. Of 112 samples submitted, 61 (i.e. about 54 per cent.) gave numbers below 24; 52 below 23; 34 below 22; 20 below 21; and 5 below 20. The lowest number obtained was 19·5. Further, as all the samples giving numbers below 24 were taken on dates between 26th October and 6th February inclusive, and as 75 samples in all were taken during this period, this shows that over 81 per cent. of these samples were below standard. Obviously the 24 limit is unsatisfactory for this particular time of the year.

Polenske Figure.—For pure butter the number is small and varies with the Reichert-Wollny number, from about 2·0 when the latter is 24 to about 3·2 with a Reichert-Wollny number of 32. In the samples under review the variations were from 1·0 to 2·36.

Koettstorfer Test (Potash Absorption, i.e. milligrams of caustic potash required to saponify one gram of fat).—Varies as a rule from 218 to 235 for pure butter. In these samples variations were from 220·2 to 234·9.

Ave-Lallemant Test (Baryta Value).—With pure butters the value is stated to be always negative, varying from −23·8 to −0·7 and averaging −9·6. In the samples under test here figures varying from −25·0 to +10·4 were obtained, 20 samples giving positive results.

Zeiss Butyro-refractometer.—Readings taken at 30° C. Samples tested varied from 47·3 to 51·3, while the limits given for pure butter are from 46·8 to 51·3.

At the Clonakilty Agricultural School the cows were, perhaps, more liberally fed than is usually the case in the south-west districts. Up to November 27th, in addition to pasture, they were given a mixture of rape and cabbage and a small ration of equal parts of palm-nut meal and crushed oats. After November 27th swedes were substituted for rape and cabbage. The cows were housed on November 21st, but allowed out daily from nine to four. For the investigation six cows were selected which had calved on various dates between April 28th and July 6th. All the animals were therefore well advanced in their lactation periods when the test commenced. The first sample of butter taken was on October 23rd, and gave a Reichert-Wollny number of 28·0. Thereafter samples were received at intervals of one week until January 29th. The Reichert-Wollny number fell as the period advanced and reached its lowest (24·0) on December 4th, after which it began

to rise. It would thus appear that the effect of advancing lactation in causing depression of the Reichert-Wollny number can, to some extent, be counteracted by liberal treatment of the cows as regards feeding and shelter.

In my previous work for the Department similar results were obtained, it being shown that where winter dairying was practised, and the cows better fed, etc., the number did not fall below 24. In these cases, however, the cows were not all calved in the spring of the year.

The following are the limits obtained in the tests on the Clonakilty samples:—

Reichert-Wollny number varied from	.	.	24·0	to 28·6
Polenske figure	„	„ .	1·58	„ 3·0
Ave-Lallemant figure	„	„ .	−16·0	„ −2·6
Koettstorfer figure	„	„ .	233·5	„ 239·8
Zeiss Refractometer reading at 30° C. varied from			45·1	„ 49·4

three results being obtained below 46·8.

It might be observed that ten of the fifteen samples gave a Koettstorfer figure above 235. In this connexion it is of interest to note that part of the ration of the cows consisted of palm-nut cake, the oil of which has a Koettstorfer value of from 243 to 255. Whether the high Koettstorfer value of the butter has any connexion with the feeding of palm-nut cake is, however, a matter with which this investigation was not concerned.

Conclusions.—This investigation has confirmed the results obtained in the former investigation and serves to emphasise further the unfairness of fixing the number 24 as the limit below which the Reichert-Wollny number should not fall without the presumption being raised that the butter is not genuine. As regards the other object of the investigation, viz. to determine whether any of the other tests might prove to be more reliable than the Reichert-Wollny test, it is our opinion that no one of them can be so considered. The results of these tests afford, however, useful information regarding the possible variations that may be looked for in them also, and suggest that analysts ought not to accept too literally the limits laid down for the various tests by writers on dairy chemistry.

It is scarcely within the scope of this paper to suggest the more general adoption of winter dairying in Ireland, but there can be little doubt that if winter dairying were the regular practice, rather than the exception, the question of pure Irish butter giving abnormal Reichert-Wollny figures would not arise. It is, however, perfectly evident that in existing circumstances samples of genuine Irish butter will be met with which will give results at variance with the recognised standards, and, having regard to the results obtained in this and in the previous investigation, such butter should not, therefore, be too readily condemned as impure, more particularly during late autumn and winter, when it is probably the produce of cows nearing the end of their lactation period.

I wish to express my indebtedness to Mr. Poole Wilson and his colleagues in the Department's Creamery Section whose co-operation enabled this investigation to be carried out. Thanks are also due to Messrs. P. Whelan, A.R.C.Sc.I., A.I.C., and R. Young, Assistants in the Chemical Advisory Division, for the painstaking care and thoroughness with which they carried out the various tests.

The following tables give detailed results of all the tests made and graphs are attached illustrating the variations in the Reichert-Wollny numbers.

In the case of Creamery IV graphs are also attached showing the results obtained in the other tests.

CREAMERY I.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
5/10/23	8/10/23	25·9	1·65	226·2	— 8·0	49·8
19/10/23	20/10/23	24·4	1·51	227·2	— 10·8	49·7
2/11/23	5/11/23	22·2	1·05	224·5	— 7·5	49·5
17/11/23	19/11/23	21·9	1·44	229·0	— 10·7	49·6
29/11/23	30/11/23	21·5	1·15	224·4	+ 1·5	49·2
15/12/23	17/12/23	21·7	1·15	223·0	+ 0·1	49·4
11/1 /24	14/1 /24	25·4	1·53	230·0	— 13·6	47·9
25/1 /24	28/1 /24	26·4	1·58	232·1	— 19·8	49·7
8/2 /24	9/2 /24	28·6	1·68	232·8	— 21·5	49·2
22/2 /24	23/2 /24	28·8	1·79	231·1	— 12·7	48·9

CREAMERY II.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
8/10/23	9/10/23	26·1	1·57	235·0	— 8·6	47·3
22/10/23	24/10/23	25·0	1·38	230·7	— 9·3	48·3
5/11/23	6/11/23	23·3	1·94	229·3	— 3·6	50·0
19/11/23	20/11/23	21·2	1·30	228·6	— 7·9	49·9
3/12/23	4/12/23	19·9	1·44	223·0	+ 0·9	49·0
17/12/23	18/12/23	20·5	1·16	220·9	— 2·6	48·5
7/1 /24	8/1 /24	20·8	1·37	222·3	+ 1·8	50·0
2/2 /24	4/2 /24	26·0	1·52	228·6	— 9·8	49·3
11/2 /24	12/2 /24	24·7	1·47	224·4	— 6·8	50·0
23/2 /24	25/2 /24	25·8	1·26	225·1	— 2·0	49·8

CREAMERY III.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
5/10/23	11/10/23	25·8	1·55	225·8	+ 0·2	50·0
19/10/23	22/10/23	25·8	1·95	231·4	— 10·7	50·0
2/11/23	5/11/23	22·1	1·51	232·1	— 9·1	49·0
16/11/23	19/11/23	22·4	1·22	226·5	— 3·0	49·9
30/11/23	3/12/23	22·3	1·44	224·4	— 0·2	49·0
14/12/23	17/12/23	21·9	1·15	225·1	— 4·4	48·7
4/1 /24	7/1 /24	24·1	1·16	227·9	— 0·1	49·6
21/1 /24	22/1 /24	27·6	1·74	230·0	— 12·0	47·9
1/2 /24	4/2 /24	27·2	1·53	230·7	— 20·3	48·1
15/2 /24	16/2 /24	30·0	1·68	231·4	— 23·2	49·2
29/2 /24	3/3 /24	30·8	1·68	228·6	— 14·8	49·0

CREAMERY IV.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
9/10/23	11/10/23	25·7	1·59	230·0	— 6·2	49·3
23/10/23	24/10/23	25·0	1·52	228·6	— 6·3	48·5
7/11/23	8/11/23	22·2	1·58	227·2	— 1·6	49·8
20/11/23	21/11/23	21·0	1·15	225·8	— 3·2	49·9
5/12/23	7/12/23	20·4	1·30	222·3	+ 6·8	48·0
19/12/23	21/12/23	20·8	1·21	222·3	+ 0·2	50·2
23/1 /24	25/1 /24	25·2	1·26	227·2	— 9·3	48·7
6/2 /24	7/2 /24	23·7	1·26	223·0	— 7·3	49·1
20/2 /24	22/2 /24	25·2	1·26	222·3	— 11·3	50·0
5/3 /24	6/3 /24	30·0	2·36	225·8	— 10·3	49·1

CREAMERY V.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
11/10/23	12/10/23	26·4	1·74	225·1	— 7·9	49·0
26/10/23	29/10/23	24·5	1·58	228·6	— 6·4	49·8
7/11/23	12/11/23	21·7	2·00	224·4	— 1·8	48·8
23/11/23	24/11/23	20·6	1·15	225·8	— 7·3	49·3
7/12/23	10/12/23	21·6	1·44	225·8	+ 4·4	49·9
21/12/23	24/12/23	22·7	1·46	227·9	— 5·0	49·4
4/1 /24	7/1 /24	22·5	1·84	228·4	— 8·4	49·4
21/1 /24	24/1 /24	25·1	1·32	227·2	— 8·1	49·4
28/1 /24	30/1 /24	25·2	1·16	228·6	— 11·4	48·2
11/2 /24	13/2 /24	26·7	1·53	227·2	— 22·1	49·3
25/2 /24	29/2 /24	27·5	1·26	224·4	— 13·3	49·2

CREAMERY VI.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
12/10/23	15/10/23	25·4	1·74	227·2	— 4·6	50·9
26/10/23	27/10/23	23·7	1·53	226·0	— 9·5	49·3
9/11/23	12/11/23	21·2	1·41	227·5	— 6·3	48·7
23/11/23	26/11/23	20·3	1·15	227·2	+ 1·3	48·3
7/12/23	8/12/23	19·8	1·15	220·9	+ 10·4	48·3
21/12/23	24/12/23	19·8	1·05	223·0	+ 8·3	51·3
11/1 /24	14/1 /24	21·5	1·26	223·7	— 2·5	50·2
26/1 /24	28/1 /24	23·7	1·21	225·8	— 5·4	49·3
9/2 /24	11/2 /24	25·1	1·21	226·5	— 14·6	49·5
22/2 /24	23/2 /24	25·8	1·00	226·7	— 13·2	49·8

Creamery VII.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
13/10/23	15/10/23	25·9	1·82	229·3	— 2·0	49·2
27/10/23	30/10/23	22·4	2·10	227 2	— 6·2	50·1
10/11/23	13/11/23	22·7	1·58	226·1	— 10·0	49·0
23/11/23	26/11/23	21·0	1·30	226·4	— 13·3	49·5
7/12/23	8/12/23	20·6	1·29	220·2	+ 9·7	50·0
18/12/23	20/12/23	21·0	1·16	223·0	— 5·6	48·4
5/1 /24	7/1 /24	22·0	1·58	225·1	+ 1·9	49·4
23/1 /24	25/1 /24	23·6	1·37	226·5	— 10·5	50·1
31/1 /24	1/2 /24	24·5	1·32	224·4	— 6·8	49·5
16/2 /24	17/2 /24	25·5	1·26	225·8	— 12·0	49·6
1/3 /24	4/3 /24	27·2	1·26	222·3	— 13·7	49·8

Creamery VIII.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
13/10/23	15/10/23	24·2	1·40	227·9	— 3·5	48·1
29/10/23	31/10/23	23·9	1·50	232·3	— 10·3	49·5
12/11/23	14/11/23	21·6	1·58	226·8	— 6·6	49·2
3/12/23	4/12/23	21·4	1·38	223·7	— 2·5	48·5
12/12/23	13/12/23	19·5	1·15	221·6	+ 6·9	48·7
29/12/23	31/12/23	21·5	1·26	225·8	+ 1·9	48·6
7/1 /24	8/1 /24	22·6	1·27	225·8	— 10·0	48·6
24/1 /24	25/1 /24	23·2	1·37	225·1	— 4·4	49·3
4/2 /24	5/2 /24	25·0	1·16	225·8	— 20·0	50·1
18/2 /24	19/2 /24	26·4	1·31	227·2	— 20·6	49·6
5/3 /24	6/3 /24	28·3	1·47	229·8	— 18·7	49·5

Creamery IX.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
13/10/23	15/10/23	26·6	1·67	231·4	— 7·9	49·1
29/10/23	31/10/23	22·4	1·34	230·7	— 9·4	48·5
12/11/23	13/11/23	23·8	1·11	230·7	— 13·7	50·0
26/11/23	27/11/23	20·2	1·08	226·1	— 12·4	48·0
10/12/23	11/12/23	20·7	1·15	225·1	+ 4·7	50·0
22/1 /24	24/1 /24	22·8	1·74	227·9	— 1·4	48·7
8/2 /24	9/2 /24	25·9	1·37	225·8	— 25·1	48·2
19/2 /24	20/2 /24	27·3	1·79	225·8	— 7·0	48·8

CREAMERY X.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
15/10/23	16/10/23	25·3	1·61	227·9	— 3·0	49·1
29/10/23	31/10/23	22·5	1·71	226·5	— 3·3	50·5
12/11/23	14/11/23	21·8	1·22	226·5	— 5·4	50·2
3/12/23	4/12/23	20·3	1·15	222·3	+ 5·9	48·3
10/12/23	11/12/23	20·3	1·08	222·3	+ 6·8	48·5
29/12/23	31/12/23	21·3	1·37	224·4	— 0·5	48·9
11/1 /24	12/1 /24	22·9	1·37	227·2	— 4·6	49·1
25/1 /24	28/1 /24	25·9	1·26	231·4	— 21·1	48·8
4/2 /24	6/2 /24	26·9	1·58	229·3	— 16·8	48·6
18/2 /24	19/2 /24	27·7	1·26	228·6	— 15·0	49·1
3/3 /24	4/3 /24	28·7	1·58	226·5	— 18·7	49·3

CREAMERY XI.

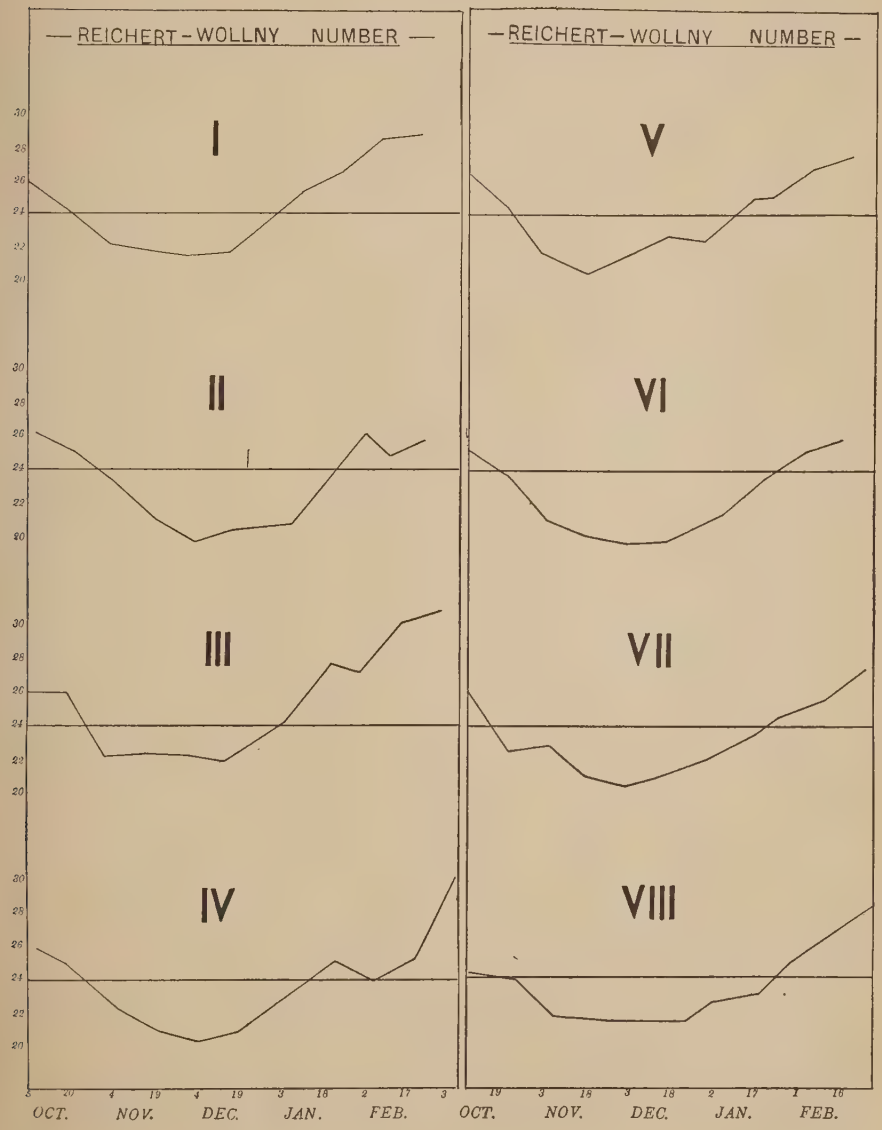
Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
17/10/23	22/10/23	26·1	1·73	229·7	— 9·4	49·7
31/10/23	2/11/23	22·9	1·65	233·5	— 11·8	49·3
13/11/23	15/11/23	22·6	1·30	228·6	— 15·9	49·9
28/11/23	30/11/23	20·3	1·30	225·8	— 7·3	49·0
12/12/23	13/12/23	19·8	1·37	223·0	— 1·5	49·1
9/1 /24	14/1 /24	20·1	1·26	221·6	+ 3·6	48·9
1/2 /24	4/2 /24	23·6	1·36	228·6	+ 1·8	48·5
15/2 /24	17/2 /24	25·1	1·26	225·1	— 11·0	50·5
29/2 /24	3/3 /24	25·7	1·31	225·8	— 16·5	50·1

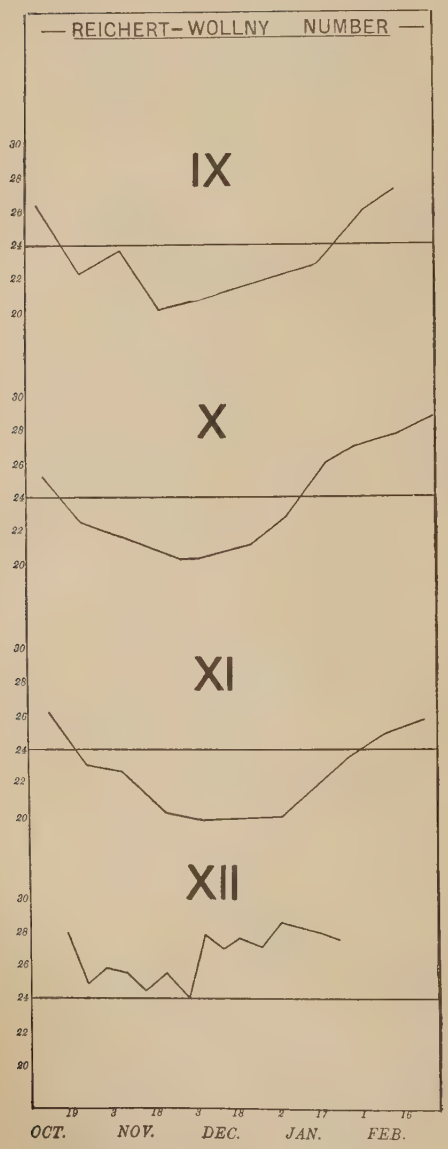
XII.—CLONAKILTY AGRICULTURAL SCHOOL.

Date of sampling.	Sample received.	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
23/10/23	25/10/23	28·0	2·02	233·5	— 16·1	48·5
30/10/23	31/10/23	24·7	2·29	234·2	— 13·4	48·9
6/11/23	7/11/23	25·8	1·80	233·5	— 11·8	48·6
13/11/23	14/11/23	25·5	2·09	237·0	— 16·0	49·4
20/11/23	21/11/23	24·5	1·58	234·2	— 13·6	48·7
27/11/23	28/11/23	25·7	1·73	234·2	— 2·6	48·0
4/12/23	5/12/23	24·0	2·60	235·6	— 3·2	45·1
11/12/23	13/12/23	27·9	2·16	238·4	— 8·7	47·0
18/12/23	20/12/23	26·8	1·68	237·7	— 11·8	48·6
24/12/23	31/12/23	27·7	2·53	235·6	— 6·5	47·8
31/12/23	3/1 /24	27·2	2·26	236·3	— 11·6	47·7
8/1 /24	9/1 /24	28·6	2·10	238·4	— 12·8	47·4
15/1 /24	16/1 /24	28·2	2·47	236·3	— 14·9	47·5
22/1 /24	24/1 /24	27·9	3·00	239·8	— 14·7	46·3
29/1 /24	30/1 /24	27·6	2·52	239·8	— 15·0	45·7

The following results obtained from two samples of margarine and from mixtures of these with butter, though not actually in the scheme of this investigation, are nevertheless interesting from the public analyst's point of view:—

	Reichert Wollny number.	Polenske number.	Koettstorfer value.	Ave-Lallemant figure.	Zeiss reading at 30° C.
Butter No. 57 (7/12/23)	19·8	1·15	220·9	+ 10·4	48·3
Butter No. 111 (15/2/24)	30·0	1·68	231·4	— 23·2	49·2
Margarine No. 1 ...	0·6	0·53	195·6	+ 26·8	57·8
Margarine No. 2 ...	8·0	7·68	236·9	+ 27·9	44·9
Mixture— 75 p.c. Butter No. 57; 25 p.c. Marg. No. 1 ...	14·9	1·0	214·6	+ 9·1	51·5
Mixture— 75 p.c. Butter No. 57; 25 p.c. Marg. No. 2 ...	16·9	2·42	225·1	+ 8·7	49·2
Mixture— 75 p.c. Butter No. 111; 25 p.c. Marg. No. 1 ...	22·2	1·06	221·6	— 8·7	50·1


— REICHERT – WOLLNY NUMBER —
I
II
III
IV
V
VI
VII
VIII
OCT.
NOV.
DEC.
JAN.
FEB.


— REICHERT-WOLLNY NUMBER —
IX
X
XI
XII
OCT.
NOV.
DEC.
JAN.
FEB.

IV
Reichert Wollney Number
Polenske Number
Koettstorfer Value
Ave-Lellamant Fig.
Zeiss Reading at 30°C
OCT.
NOV.
DEC.
JAN.
FEB.

No. 6.

THE THEORY OF VARIATION OF FLOW IN PIPE-LINES WITH SURGE CHAMBERS CONSEQUENT ON VARIATION OF LOAD ON HYDRAULIC TURBINES OPERATED THEREFROM.

By H. H. JEFFCOTT.

(Read December 16, 1924. Printed March 12, 1925.)

(1) In hydro-electric power installations the following elements are ordinarily present:—(*a*) A reservoir in which the water-level remains sensibly constant during short periods of time, (*b*) a pipe-line or tunnel leading from the reservoir to (*c*) a surge chamber or stand pipe or a forebay, (*d*) a penstock or pipe-line leading from the forebay to the turbine.

Under steady conditions of load the level of the water in the surge chamber or forebay will remain constant, and the velocities of flow in the tunnel and pipe-line will be inversely proportional to their areas, since the quantity of water discharged per second through the one is equal to that discharged through the other.

When, however, the load on the turbine is increased the turbine gates are opened wider by governor regulation. This leads to an increased discharge through the pipe-line, to a transient fall of level in the surge chamber, and to an increased velocity of flow in the tunnel. After some oscillation of level in the surge chamber and of velocities in the pipe-lines, a steady condition is again reached in course of time, due to the damping effect of friction, unless the governor action synchronises with a natural period and sustains resonant oscillation.

It is also to be observed that the effective head at the surge chamber is likewise subject to fluctuations. For on the opening of the gates the increased velocity in the pipe-line requires the expenditure of some of the gross head on accelerating the water content of the chamber. In the tunnel, too, a portion of the gross head is used to accelerate the water within it. While this acceleration is being set up in the tunnel, the water-level in the surge chamber falls, thus, not only providing some of the increased discharge of the turbine, but also increasing the difference of gravitational head between the two ends of the tunnel, which thus contributes to the accelerating head used in it.

(2) It is proposed in this paper to investigate in an approximate manner the theory of these fluctuations of head and velocity. To this end the water will be regarded as incompressible, and the pipe-line and tunnel as rigid; for the time of opening or closing of the turbine gates is usually large compared with the time required for a pressure wave to be transmitted through the water throughout the length of the pipe-line and tunnel. Further, it will be convenient in some of the investigations to regard the frictional loss of head at the walls of the pipes as sufficiently small, compared with the total heads considered, to be neglected in obtaining a first approximation to the solution.

The equations governing the motion of the water are difficult of solution in general terms. In sections (7) to (10) an analytical method of arriving at

an approximate solution of the equations is given, and is applicable when the change of velocity in the pipe-line leading to the turbine is given as a function of the time. This method of successive approximation consists in omitting small quantities at first, and solving the simplified equations for a first approximation to the quantities sought. The differences between these results and the true values of the quantities may then be solved for, the complete equations being now simplified by substituting in the small terms previously omitted the values obtained as a result of the first approximation. In this way a second approximation is obtained.

In sections (11) to (19) an inverse method of solution is outlined, and approximate results are recorded giving the solution of the equations when the given function of the time which expresses the variation of level in the surge chamber is of certain types.

Sections (20) and (21) contain a tabular arithmetical method of differences leading to approximate solution; and in sections (22) and (23) the corresponding method of graphical computation is outlined.

It is to be noted that the acceleration of the water in the surge chamber may not in general be regarded as negligible. At the outset of the motion the water in the surge chamber is at rest, and stands at a slightly lower level than that of the reservoir, owing to the friction loss in the tunnel. A change of gate opening leads to a change of head in the water at the foot of the surge chamber, by reason of the changed demand for water at the turbines. The difference of head thus brought about, acting on the water in the surge chamber, gives acceleration to it, just as the corresponding difference of head gives rise to acceleration of motion of the water in the tunnel. In fact, the accelerative heads on the water in the surge chamber and in the tunnel are then entirely comparable, and although the mass of water in the surge chamber is small compared with that in the tunnel, the change of velocity produced in it by change of head is correspondingly greater.

A little later gravitational head is set up by reason of the rise or fall of level in the surge chamber (while the level in the reservoir remains sensibly constant), so that this additional term becomes effective in retarding or increasing the velocity in the tunnel as the case may be. Thus both the accelerative and gravitational terms relative to the surge chamber (in addition to the small effect of friction) are then influential in determining the head causing acceleration of the water in the tunnel.

In applications where the conditions lead to rapid change of velocity in the surge chamber (e.g. sudden opening or closing of the gates), the acceleration therein is comparatively large and the corresponding term in the equation is important. This is also the case when the area of the surge chamber is relatively small. Under conditions, however, in which the acceleration in the surge chamber is known to be always small (e.g. with slow operation of the gates), the corresponding term may remain sufficiently small throughout the motion to be omitted in approximate calculation.

It is proposed in this paper to deal with the general case, so that all applications may be included. The solution of the equations is considerably simplified when this accelerative term may be omitted, and the results corresponding to this hypothesis are indicated in each case.

(3) Fig. 1 represents diagrammatically the elements of the problem. Let the areas of the tunnel, pipe-line, and surge chamber be A, B, and P sq. ft.; and the length of tunnel and pipe be L and l ft. respectively. Let the surge chamber be of cylindrical form and be connected with the pipe-line by an

opening of area P' in a diaphragm near the base of the surge chamber, where P' is less than P. If the surge chamber were not of uniform cross-sectional area throughout its height, but of varying area, then P would not be constant, but would be a function of the depth of water within it. Let the water-level in the reservoir, which is regarded as constant during the periods considered, be H ft. above datum, which is the tail race level. Let the water-level in the surge chamber or stand pipe at time t seconds from the commencement of the change from steady conditions be y ft. above the junction of the tunnel and pipe-line with the stand pipe, which junction is s ft. above datum. Let the effective head at the turbine at time t be h ft. above datum. Let the velocities in the tunnel and pipe-line at time t be u and v ft. per second respectively, both being positive in the direction of flow from reservoir to turbines. Let the frictional loss of head at unit velocity in the tunnel, pipe-line, and stand pipe be μ, μ', and $\mu''y$ respectively; so that the losses of head are approximately μu^2, $\mu' v^2$, and $\mu'' y \left(\frac{dy}{dt}\right)^2$ respectively. If, for greater exactness in exceptional applications, the losses of head be taken as μu^n, $\mu' v^n$, and $\mu'' y \left(\frac{dy}{dt}\right)^n$, where n is slightly less than 2, the methods given in the paper may still be employed in certain cases. Also let the loss of head due to the constriction in the diaphragm be $\lambda\left(\frac{dy}{dt}\right)^2$ approximately.

If the forebay or surge chamber is arranged so that the velocity u of the inflowing water from the tunnel is destroyed in disturbed motion, then there is a corresponding sacrifice of head equal to $\frac{u^2}{2g}$; and likewise we will suppose there are similar losses of head in the penstock of $\frac{v^2}{2g}$, and in the surge chamber of $\frac{1}{2g}\left(\frac{dy}{dt}\right)^2$. If the conditions in any particular application are such that one of these losses does not occur, the corresponding term in the equations must be omitted.

Let suffixes 0 indicate the values of the quantities at $t = 0$, or the commencement of regulation, and suffixes 1 correspond to $t = t_1$, or the completion of the gate regulation.[1] The atmospheric pressure is regarded as being the same at the reservoir and at the surge chamber.

We can now write down certain relations that must obtain between the various quantities for a cylindrical surge chamber, the operations being supposed to start from a condition of steady flow.

At the commencement, for a steady condition of flow—

$$g(H_0 - s) = gy_0 + (g\mu + \tfrac{1}{2})u_0^2. \qquad (1)$$

$$gy_0 = g(h_0 - s) + (g\mu' + \tfrac{1}{2})v_0^2. \qquad (2)$$

$$Au_0 = Bv_0. \qquad (3)$$

[1] On pages 64 to 66 suffixes 1 have a different meaning.

During the regulation, at time t, if $\frac{Y}{g} + s$ is the head in the water at the position where the surge chamber joins the pipe-line,

$$g(H - s) \mp (g\mu + \tfrac{1}{2})u^2 - L\frac{du}{dt} = gy \pm (g\mu''y + g\lambda + \tfrac{1}{2})\left(\frac{dy}{dt}\right)^2 + y\frac{d^2y}{dt^2} = Y. \quad (4)$$

$$Y = gy \pm (g\mu''y + g\lambda + \tfrac{1}{2})\left(\frac{dy}{dt}\right)^2 + y\frac{d^2y}{dt^2} = g(h - s) + (g\mu' + \tfrac{1}{2})v^2 + l\frac{dv}{dt}. \quad (5)$$

$$P\frac{dy}{dt} = Au - Bv. \quad (6)$$

$$H = H_0. \quad (7)$$

Water horse-power supplied to turbine $= \frac{Bhv}{8{\cdot}83}$. (8)

The upper (or lower) sign in equations (4) and (5) is to be taken for the terms in

$$u^2 \text{ and } \left(\frac{dy}{dt}\right)^2 \quad \text{according as } u \text{ and } \frac{dy}{dt}$$

are positive (or negative) respectively. It is to be noted that v is always positive in practice.

For ordinary conditions of roughness

$$\mu = {\cdot}0004\frac{L}{D}, \quad \mu' = {\cdot}0004\frac{l}{d}, \quad \text{and} \quad \mu'' = {\cdot}0001\frac{f}{P},$$

where D and d are the diameters of the tunnel and pipe-line respectively, and f is the perimeter of cross-section of the surge chamber or forebay. Also with regard to the loss of head at the aperture in the diaphragm we have approximately

$$\lambda = \frac{1}{2g}\left\{\sqrt{2{\cdot}62\frac{P^2}{P'^2} - 1{\cdot}62} - 1\right\}^2.$$

In the undamped case where there is no diaphragm $P' = P$, and $\lambda = 0$.

If y is known (or can be assumed) as a function of t, equation (4) gives u as a function of t. Thus (6) gives v as a function of t, and (5) gives h also as a function of t. Otherwise if v is given as a function of t, the equations (4) and (6) give u and y as functions of t, while (5) gives h.

(4) These equations control the motion while regulation is being effected, i.e. up to $t = t_1$. Thereafter similar equations will hold good, but v will in general be a different function of t, and will gradually attain a steady value v_1. Or the regulation may be such that the whole change of v to its final steady value will proceed according to a single function of t.

(5) In the particular case when the load is entirely thrown off the turbine, and the gates are rapidly closed, the amplitude of the first oscillation of the water thereafter in the tunnel and surge chamber can be readily determined, if the loss of energy due to friction and damping may be regarded as negligible. Thus let the water in the tunnel be moving at constant velocity u_0 and that in the surge chamber be at rest (so that, neglecting friction, it stands therein at the reservoir level), and let the gates be suddenly closed.

To obtain with sufficient exactness the kinetic energy with which the whole mass of water commences to surge forward we may equate the momentum of the water in the tunnel before its impact with the stationary water in the surge chamber to the momentum of the whole mass of water after impact. Hence

$$\rho A L u_0 = \rho A L u'_0 + \rho P y_0 \left(\frac{dy}{dt}\right)_0, \quad \text{where} \quad u'_0 \text{ and } \left(\frac{dy}{dt}\right)_0$$

are the velocities in the tunnel and surge chamber respectively after impact. Also

$$P\left(\frac{dy}{dt}\right)_0 = A u'_0.$$

Hence the kinetic energy of the water after impact is

$$\tfrac{1}{2}\rho A L u'^2_0 + \tfrac{1}{2}\rho P y_0 \left(\frac{dy}{dt}\right)^2_0, \quad \text{or} \quad \tfrac{1}{2}\rho \cdot \frac{A(PL + Ay_0)L^2}{P^2(L + y_0)^2} u_0^2.$$

Equating this to the change of potential energy when the water comes to rest at a level y'': $\tfrac{1}{2}\rho g P (y'' - H + s)^2$ we find

$$y'' - H + s = \frac{L u_0}{P(L + y_0)} \sqrt{\frac{A(PL + Ay_0)}{g}}. \tag{9}$$

This gives y'', or the height in feet to which the surge chamber must be carried to avoid overflow consequent on surge oscillation when the load is quickly thrown off, the velocity in the tunnel being initially u_0 ft. per sec. The rise above steady level due to the surge is $y'' - H + s$.

On the return oscillation the potential energy thus stored in the surge chamber is utilised in giving velocity to the water in the tunnel and surge chamber. Part of this energy is carried into the reservoir and rendered unavailable for aiding the further oscillations, which, in consequence of this loss and the loss due to friction and damping, gradually diminish in amplitude.

(6) In order that the turbine shall give a greater output when the gates are opened, consequent on an increased demand being made upon it, it is necessary that the time t_1 of opening the gates shall not be too small. This value of t_1 may be found from the consideration that hv must increase and must, therefore, always exceed $h_0 v_0$ throughout the regulation. If t_1 be less than this limiting value, the reduction of the effective head h due to the acceleration of the water in the pipe is in greater proportion than the increase of the velocity, and hv becomes less instead of greater.

(7) If we are given the law of change of velocity of water in the pipe-line as a function of the time, consequent on the opening of the turbine gates, we have v as a function of t. Substituting for v in equation (6) we get a relation between u, y, and t; and with the aid of equation (4), u and y may be found as functions of t.

The solution of these equations is, in general, complicated. A method of approximation may, however, be employed based on the omission at first of unimportant quantities, and thus an approximate solution be obtained. A second approximation may then be made, if desired. To this end the differences between the true values of the quantities sought and the values just obtained may be determined approximately from the general equations, in which, however, the values resulting from the first approximation are substituted in the

unimportant terms previously omitted. In like manner a third approximation may be obtained if required.

The terms representing loss of head due to friction and velocity head are usually small in comparison with the other terms involved in the equations, and accordingly for a first approximation we may omit the terms containing $g\mu + \frac{1}{2}$, $g\mu' + \frac{1}{2}$, and $g\mu''y + g\lambda + \frac{1}{2}$. Further, as

$$\{(H - s) - y\} \frac{d^2y}{dt^2}$$

is small in most applications, we may put

$$(H - s)\frac{d^2y}{dt^2} \quad \text{for} \quad y\frac{d^2y}{dt^2}$$

in a first approximation.

We thus find that equations (4) and (5) become

$$g(H - s) - L\frac{du}{dt} = gy + (H - s)\frac{d^2y}{dt^2}. \tag{4a}$$

$$gy + (H - s)\frac{d^2y}{dt^2} = g(h - s) + l\frac{dv}{dt}. \tag{5a}$$

Eliminating u between equations (4a) and (6) we find

$$\{A(H - s) + PL\}\frac{d^2y}{dt^2} + Agy - Ag(H - s) + BL\frac{dv}{dt} = 0. \tag{10}$$

In this equation v is supposed to be a given function of t.
For convenience put

$$p = \sqrt{\frac{Ag}{A(H - s) + PL}}, \quad \text{and} \quad r = A(H - s) + PL.$$

The complementary function y_1 is obtained as the solution of

$$r\frac{d^2y}{dt^2} + Agy = 0,$$

so that

$$y_1 = c_1 \sin(pt + c_2), \tag{11}$$

where c_1 and c_2 are the arbitrary constants of integration.

We have next to find a particular integral y_2—the simpler the better—of equation (10). As v is known y_2 may usually be found.

The following values may be seen to satisfy equation (10):—

If $v = \alpha + \beta t + \gamma t^2 + \delta t^3$, $y_2 = H - s - \frac{BL\beta}{Ag} + \frac{6BLr\delta}{A^2g^2} - \frac{2BL\gamma}{Ag}t - \frac{3BL\delta}{Ag}t^2$. (12)

If $v = a \sin \beta t + \gamma$, $y_2 = H - s + \frac{BLa\beta}{r\beta^2 - Ag}\cos \beta t$. (13)

If $v = ae^{\beta t} + \gamma$, $y_2 = H - s - \frac{BLa\beta}{r\beta^2 + Ag}e^{\beta t}$. (14)

If $v = ae^{\beta t} \sin \gamma t + \delta$,

$$y_2 = H - s - \frac{BLae^{\beta t}\{\{Ag + r(\beta^2 + \gamma^2)\}\beta \sin \gamma t + \{Ag - r(\beta^2 + \gamma^2)\}\gamma \cos \gamma t\}}{A^2g^2 + 2Agr(\beta^2 - \gamma^2) + r^2(\beta^2 + \gamma^2)^2}. \tag{15}$$

The complete solution of the approximate equations is $y_1 + y_2$, or say y'.

(8) We have thus obtained a first approximation to the value of y. If the omitted terms always exercise a negligible influence in the equations, this solution is sufficient. We may then proceed to find the values of u and h from equations (6) and (5*a*) respectively.

Thus if $v = a \sin \beta t + \gamma$, we find $Au = Pc_1 p \cos(pt + c_2) + (Ba - Pq\beta) \sin \beta t + B\gamma$, and $Agh = AgH + c_1 PLp^2 \sin(pt + c_2) - \{(Al + BL)a - PLq\beta\}\beta \cos \beta t$, where

$$q = \frac{BLa\beta}{r\beta^2 - Ag}.$$

(9) If, however, this first approximation for y is not sufficiently exact we may proceed to find a second approximation in the following manner. Suppose the exact value y to differ from the value $y' = y_1 + y_2$ by a small amount η, so that $y = y' + \eta$. Replace y in the original equations (4), (5), (6) by $y' + \eta$ and we obtain new equations in η instead of in y. In these new equations we will, as a second approximation, include the friction and velocity head terms so far as they depend on y'.

Thus equations (4) and (6) become

$$g(H - s) \mp (g\mu + \tfrac{1}{2})u_1^2 - L\frac{du}{dt} = g(y' + \eta) \pm (g\mu''y' + g\lambda + \tfrac{1}{2})\left(\frac{dy'}{dt}\right)^2 + (y' + \eta)\frac{d^2(y' + \eta)}{dt^2} \qquad (4b)$$

and

$$P\frac{dy'}{dt} = Au_1 - Bv. \qquad (6a)$$

We have now to eliminate u and u_1 between (4*b*), (6), and (6*a*). We thus find

$$A^2 g(H - s) \mp (g\mu + \tfrac{1}{2})\left\{P\frac{dy'}{dt} + Bv\right\}^2 - ABL\frac{dv}{dt} - APL\frac{d^2y'}{dt^2} - APL\frac{d^2\eta}{dt^2}$$

$$= A^2 gy' \pm A^2(g\mu''y' + g\lambda + \tfrac{1}{2})\left(\frac{dy'}{dt}\right)^2 + A^2 y'\frac{d^2y'}{dt^2} + A^2 g\eta + A^2\eta\frac{d^2y'}{dt^2} + A^2(y' + \eta)\frac{d^2\eta}{dt^2}.$$

Further, if we put as before $H - s$ for $y' + \eta$ in certain unimportant terms and omit negligible quantities we find

$$Ar\frac{d^2\eta}{dt^2} + A^2 g\eta + A^2\{y' - (H - s)\}\frac{d^2y'}{dt^2} \pm (g\mu + \tfrac{1}{2})\left\{P\frac{dy'}{dt} + Bv\right\}^2 \pm A^2\left\{g\mu''(H - s) + g\lambda + \tfrac{1}{2}\right\}\left(\frac{dy'}{dt}\right)^2 = 0, \quad (16)$$

since the value y' makes

$$r\frac{d^2y'}{dt^2} + Agy' - Ag(H - s) + BL\frac{dv}{dt} = 0.$$

Equation (16) is of the form

$$a\frac{d^2\eta}{dt^2} + b\eta + \phi = 0,$$

where ϕ is a known function of t. It may be solved in similar manner to equation (10).

The complementary function is

$$\eta_1 = c_3 \sin (pt + c_4) \qquad (17)$$

where c_3 and c_4 are the arbitrary constants of integration.

A particular integral η_2 may usually be obtained when the value of v as a function of t is known.

Thus if $v = a \sin \beta t$ we have

$$y' = c_1 \sin (pt + c_2) + (H - s) + q \cos \beta t.$$

Substituting in equation (16), we require to find a particular integral of

$$Ar\frac{d^2\eta}{dt^2} + A^2g\eta - A^2 \{c_1 p^2 \sin (pt + c_2) + q\beta^2 \cos \beta t\} \{c_1 \sin (pt + c_2) + q \cos \beta t\}$$

$$\pm (g\mu + \tfrac{1}{2}) \{Pc_1 p \cos (pt + c_2) - Pq\beta \sin \beta t + Ba \sin \beta t\}^2$$

$$\pm A^2 \{g\mu'' (H - s) + g\lambda + \tfrac{1}{2}\} \{c_1 p \cos (pt + c_2) - q\beta \sin \beta t\}^2 = 0. \qquad (18)$$

A particular integral of equation (18) is

$$\eta_2 = \frac{a'}{Ag} + \frac{b'}{3Ag} \cos 2 (pt + c_2) + \frac{c'}{4r\beta^2 - Ag} \cos 2\beta t$$

$$+ \frac{d'}{r (p + \beta)^2 - Ag} \sin \{(p + \beta) t + c_2\} + \frac{e'}{r (p - \beta)^2 - Ag} \sin \{(p - \beta) t + c_2\}. \qquad (19)$$

where

$$a' = \tfrac{1}{2}A (c_1^2 p^2 + q^2\beta^2) \mp \frac{g\mu + \frac{1}{2}}{2A} \{c_1^2 P^2 p^2 + (Ba - Pq\beta)^2\}$$

$$\mp \tfrac{1}{2}A \{g\mu'' (H - s) + g\lambda + \tfrac{1}{2}\} (c_1^2 p^2 + q^2\beta^2).$$

$$b' = \frac{c_1^2 p^2}{2A} [A^2 \pm P^2 (g\mu + \tfrac{1}{2}) \pm A^2 \{g\mu'' (H - s) + g\lambda + \tfrac{1}{2}\}].$$

$$c' = -\frac{1}{2A} [A^2 q^2\beta^2 \pm (g\mu + \tfrac{1}{2}) (Ba - Pq\beta)^2 \pm \{g\mu'' (H - s) + g\lambda + \tfrac{1}{2}\} A^2 q^2\beta^2].$$

$$d' = -\frac{c_1}{A} [\tfrac{1}{2}A^2 q (p^2 + \beta^2) \mp (g\mu + \tfrac{1}{2}) Pp (Ba - Pq\beta) \pm \{g\mu'' (H - s) + g\lambda + \tfrac{1}{2}\} A^2 pq\beta].$$

$$e' = -\frac{c_1}{A} [\tfrac{1}{2}A^2 q (p^2 + \beta^2) \pm (g\mu + \tfrac{1}{2}) Pp (Ba - Pq\beta) \mp \{g\mu'' (H - s) + g\lambda + \tfrac{1}{2}\} A^2 pq\beta].$$

Hence the complete solution of equation (16) is $\eta_1 + \eta_2$.

Accordingly when $v = a \sin \beta t$ we have as a second approximation to the value of y $y = y_1 + y_2 + \eta_1 + \eta_2$, where y_1, y_2, η_1, and η_2 are given respectively by equations (11), (13), (17), and (19).

The values of u and h as functions of t may be obtained from this value of y by aid of the equations (6) and (5) respectively. Corresponding equations can be derived for other given values of v in terms of t.

(10) If we confine our attention to those applications in which the method of operation of the gates ensures that the acceleration in the surge chamber is always small in its effect, the accelerative term $y\frac{d^2y}{dt^2}$ may be neglected in equations (4) and (5).

At the outset, if friction be omitted $g(H - s) = gy$, so that

$$L\frac{du}{dt} = -y\frac{d^2y}{dt^2}.$$

Accordingly $y\frac{d^2y}{dt^2}$ is not small compared with $g(H - s - y)$ which is then zero; but in this case we will suppose that its value is so small that its effect in producing change of velocity of the water in the tunnel is negligible, and that this change of velocity is almost entirely due to gravitational head developed as the gate opening proceeds.

Equation (4) now becomes

$$g(H - s) \mp (g\mu + \tfrac{1}{2})u^2 - L\frac{du}{dt} = gy \pm (g\mu''y + g\lambda + \tfrac{1}{2})\left(\frac{dy}{dt}\right)^2. \qquad (4c)$$

The solution in this case follows the lines of that given in sections (7) to (9).

Firstly, we regard the frictional terms as negligible, and we have

$$g(H - s) - L\frac{du}{dt} = gy. \qquad (4d)$$

Eliminating u between equations (4d) and (6) we obtain

$$PL\frac{d^2y}{dt^2} + Agy - Ag(H - s) + BL\frac{dv}{dt} = 0. \qquad (10a)$$

This equation is similar to equation (10) if $r' = PL$ be substituted for r. Accordingly its solution (giving a first approximation) is as given in equations (11) to (15), in which r' is substituted for r,

$$p' = \sqrt{\frac{Ag}{PL}} \text{ for } p, \text{ and } q' = \frac{BL\alpha\beta}{r'\beta^2 - Ag} \text{ for } q.$$

To obtain a second approximation, $y' + \eta$ is to be substituted for y in (4c), so that

$$g(H - s) \mp (g\mu + \tfrac{1}{2})\mu_1^2 - L\frac{du}{dt} = g(y' + \eta) \pm (g\mu''y' + g\lambda + \tfrac{1}{2})\left(\frac{dy'}{dt}\right)^2. \qquad (4e)$$

From this equation coupled with (6) and (6a) we eliminate u and u_1, and obtain

$$APL\frac{d^2\eta}{dt^2} + A^2g\eta \pm (g\mu + \tfrac{1}{2})\left(P\frac{dy'}{dt} + Bv\right)^2 \pm A^2(g\mu''y' + g\lambda + \tfrac{1}{2})\left(\frac{dy'}{dt}\right)^2 = 0, \qquad (16a)$$

since $$PL\frac{d^2y'}{dt^2} + Agy' - Ag(H - s) + BL\frac{dv}{dt} = 0.$$

The complementary function is as in section (9), but with p' written for p.

Now $y' = c_1 \sin(p't + c_2) + H - s + q'\cos\beta t$, so that we require to find a particular integral of

$$Ar'\frac{d^2\eta}{dt^2} + A^2g\eta \pm (g\mu + \tfrac{1}{2})\{c_1Pp'\cos(p't + c_2) + (B\alpha - Pq'\beta)\sin\beta t\}^2$$

$$\pm A^2\{g\mu''(H - s) + g\lambda + \tfrac{1}{2}\}\{c_1p'\cos(p't + c_2) - q'\beta\sin\beta t\} = 0. \qquad (18a)$$

A particular integral is

$$\eta_2 = \frac{a''}{Ag} + \frac{b''}{3Ag}\cos 2(p't + c_2) + \frac{c''}{4r'\beta^2 - Ag}\cos 2\beta t$$

$$+ \frac{d''}{r'(p'+\beta)^2 - Ag}\sin\{(p'+\beta)t + c_2\} - \frac{d''}{r'(p'-\beta)^2 - Ag}\sin\{(p'-\beta)t + c_2\}. \quad (19a)$$

where

$$a'' = \mp\frac{1}{2A}\Big[(g\mu + \tfrac{1}{2})\{c_1^2P^2p'^2 + (Ba - Pq'\beta)^2\} + A^2\{g\mu''(H-s) + g\lambda + \tfrac{1}{2}\}(c_1^2p'^2 + q'^2\beta^2)\Big].$$

$$b'' = \pm\frac{c_1^2p'^2}{2A}\Big[P^2(g\mu + \tfrac{1}{2}) + A^2\{g\mu''(H-s) + g\lambda + \tfrac{1}{2}\}\Big].$$

$$c'' = \mp\frac{1}{2A}\Big[(g\mu + \tfrac{1}{2})(Ba - Pq'\beta)^2 + A^2q'^2\beta^2\{g\mu''(H-s) + g\lambda + \tfrac{1}{2}\}\Big].$$

$$d'' = \pm\frac{c_1p'}{A}\Big[P(g\mu + \tfrac{1}{2})(Ba - Pq'\beta) - A^2q'\beta\{g\mu''(H-s) + g\lambda + \tfrac{1}{2}\}\Big].$$

The solution then follows as in section (9).

(11) It is sometimes simpler to adopt an inverse method of solution and to assume a suitable form for y as a function of t. To obtain simplification (as in section (7)) it is often legitimate as a first approximation to omit the terms involving $(g\mu + \frac{1}{2})$, $(g\mu' + \frac{1}{2})$, and $(g\mu''y + g\lambda + \frac{1}{2})$, as these terms are commonly small compared with the total heads involved. On this basis u is determined from equation (4), and equation (6) then gives v. When a more accurate result is required, the values of u, v, and $\frac{dy}{dt}$ thus found can be substituted in the frictional terms, and a second approximation to the values of u and v be obtained.

If the value of v as a function of t obtained by this method does not sufficiently approximate to the given law of change of velocity, a different form of function for y may be tried till a sufficiently satisfactory function for v results.

(12) As an example let us assume that, during the period of opening or closing the gates, y is a simple algebraic function of the time given by

$$y = a + bt^4. \quad . \quad . \quad . \quad (20)$$

Equation (4), with the frictional losses of head omitted, is

$$L\frac{du}{dt} = gH' - y\left(g + \frac{d^2y}{dt^2}\right), \quad (21)$$

where H' is written for $H - s$. Also write h' for $h - s$. On substituting for y and integrating we find

$$L(u - u_0) = g(H' - a)t - 4abt^3 - \tfrac{1}{5}bgt^5 - 2b^2t^6. \quad (22)$$

From equation (6)

$$L(Bv - Au_0) = gA(H' - a)t - 4b(Aa + PL)t^3 - \tfrac{1}{5}bgAt^5 - 2b^2At^6. \quad (23)$$

Then equation (5) gives h'

$$gBLh' = gBLH' - g(H' - a)(Al + BL) + 12bt^2\{a(Al + BL) + PLl\}$$
$$+ bgt^4(Al + BL) + 12b^2t^5(Al + BL). \qquad (24)$$

These equations determine the values of u, v, and h corresponding to the assumed value of y throughout the period of change of opening of the turbine gates.

The value of the constant a may be found if the initial value of y be supposed known. Thus putting $t = 0$ we find $a = y_0$.

The value of b is determined if the value of y, or of v, is known at the time t_1. In the latter case b is given by equation (23) when v_1, t_1, and y_0 are written for v, t, and a.

It is to be noted that with the foregoing assumption of y as a function of t, and neglecting the losses of head due to friction and velocity, we have, when $t = 0$,

$$y = y_0, \quad \frac{dy}{dt} = 0, \quad u = u_0, \quad v = v_0;$$

$$Au_0 = Bv_0, \quad h'_0 = y_0 = H', \quad H' - a = 0, \quad \frac{dh'}{dt} = 0.$$

From equation (8) the power supplied to the turbines at any instant t seconds from the commencement of regulation is found from the values of v and h given by equations (23) and (24). If desired, a second approximation may then be made as indicated in section (11).

(13) When the acceleration in the surge chamber may be neglected the results of section (12) become simplified.

We find

$$L\frac{du}{dt} = gH' - gy. \qquad (21a)$$

$$L(u - u_0) = -\tfrac{1}{5}gbt^5. \qquad (22a)$$

$$BL(v - v_0) = -4PLbt^3 - \tfrac{1}{5}gbAt^5. \qquad (23a)$$

$$gBL(h - H) = 12bPLlt^2 + gb(Al + BL)t^4. \qquad (24a)$$

since $a = y_0 = H'$.

(14) If we assume that y is given by

$$y = a + be^{-at}\sin\beta t. \qquad (25)$$

and that the frictional terms may be neglected, it may be shown that u is given by

$$L(u - u_1) = \frac{b^2(a^2 - \beta^2)}{4a}e^{-2at} + \frac{be^{-at}}{a^2 + \beta^2}\{g - y_0(a^2 - 3\beta^2)\}a\sin\beta t$$
$$+ \frac{be^{-at}}{a^2 + \beta^2}\{g - y_0(3a^2 - \beta^2)\}\beta\cos\beta t - \frac{b^2e^{-2at}}{4}\beta\sin 2\beta t - \frac{b^2e^{-2at}}{4}a\cos 2\beta t. \qquad (26)$$

since $a = y_0 = H'$.

Then v is given by

$$\frac{LB}{A}(v-v_1) = \frac{b^2(a^2-\beta^2)}{4a}e^{-2at} + \frac{be^{-at}}{a^2+\beta^2}\left\{g - y_0(a^2-3\beta^2) + \frac{PL}{A}(a^2+\beta^2)\right\} a\sin\beta t$$
$$+ \frac{be^{-at}}{a^2+\beta^2}\left\{g - y_0(3a^2-\beta^2) - \frac{PL}{A}(a^2+\beta^2)\right\}\beta\cos\beta t - \frac{b^2e^{-2at}}{4}\beta\sin 2\beta t$$
$$- \frac{b^2e^{-2at}}{4}a\cos 2\beta t. \qquad (27)$$

h is given by

$$\frac{BLg}{Al+BL}(h-H) = \frac{b^2(a^2-\beta^2)}{2}e^{-2at} + be^{-at}\left[g - (a^2-\beta^2)\left\{y_0 - \frac{PLl}{Al+BL}\right\}\right]\sin\beta t$$
$$- 2ba\beta e^{-at}\left\{y_0 + \frac{PLl}{Al+BL}\right\}\cos\beta t - b^2a\beta e^{-2at}\sin 2\beta t$$
$$- \tfrac{1}{2}b^2(a^2-\beta^2)e^{-2at}\cos 2\beta t. \qquad (28)$$

where u_1 and v_1 are the final values of u and v.

(15) When the acceleration in the surge chamber is omitted the results of section (14) become as follows:—

$$L(u-u_1) = \frac{gbe^{-at}}{a^2+\beta^2}(a\sin\beta t + \beta\cos\beta t). \qquad (26a)$$

$$BL(v-v_1) = \frac{be^{-at}}{a^2+\beta^2}\left[\{Ag + PL(a^2+\beta^2)\}a\sin\beta t + \{Ag - PL(a^2+\beta^2)\}\beta\cos\beta t\right] \qquad (27a)$$

$$gBL(h-H) = be^{-at}\sin\beta t\{g(Al+BL) + PLl(a^2-\beta^2)\} - 2bPLla\beta e^{-at}\cos\beta t. \qquad (28a)$$

(16) Or, again, if we assume for y the value

$$y = a + bt^2e^{-at}\sin\beta t, \qquad (29)$$

u is found to be given by

$$8La^5(a^2+\beta^2)^3(u-u_1)$$
$$= 8ba^5e^{-at}\sin\beta t[2ga(a^2-3\beta^2) + 2\{g(a^2-\beta^2) - a(a^2+\beta^2)^2\}(a^2+\beta^2)t$$
$$+ a(a^2+\beta^2)^2\{g + a(a^2+\beta^2)\}t^2]$$
$$+ 8ba^5\beta e^{-at}\cos\beta t[2g(3a^2-\beta^2) + 4ga(a^2+\beta^2)t + (a^2+\beta^2)^2\{g - a(a^2+\beta^2)\}t^2].$$
$$- b^2a^5\beta e^{-2at}\sin 2\beta t[3a^2 - \beta^2 + 4a(a^2+\beta^2)t + 2(a^2+\beta^2)^2t^2 + 2(a^2+\beta^2)^3t^4]$$
$$+ b^2a^5e^{-2at}\cos 2\beta t[a(a^2-3\beta^2) + 2(a^4-\beta^4)t + 2a(a^2+\beta^2)^2t^2$$
$$+ 4(a^2+\beta^2)^3t^3 - 2a(a^2+\beta^2)^3t^4]$$
$$- b^2(a^2+\beta^2)^3e^{-2at}[(a^2+3\beta^2)(1+2at+2a^2t^2) + 4a^3t^3(a^2+\beta^2) - 2a^4t^4(a^2-\beta^2)], \qquad (30)$$

since $a = y_0 = H'$ and u_1 is the final value of u.

Then v is obtained from this value of u in accordance with the equation

$$Bv = Au - bPte^{-at}\{2\sin\beta t - at\sin\beta t + \beta t\cos\beta t\}. \qquad (31)$$

h is obtained from equation (5) on substituting the value of v just obtained, and the assumed value of y.

(17) If the acceleration in the surge chamber be neglected the result in equation (30) is greatly simplified. It becomes—

$$\frac{L}{g\bar{b}}(u - u_1) = e^{-\alpha t}\sin\beta t\left[\frac{2\alpha(\alpha^2 - 3\beta^2)}{(\alpha^2+\beta^2)^3} + \frac{2(\alpha^2-\beta^2)}{(\alpha^2+\beta^2)^2}t + \frac{\alpha}{\alpha^2+\beta^2}t^2\right]$$

$$+ e^{-\alpha t}\cos\beta t\left[\frac{2\beta(3\alpha^2-\beta^2)}{(\alpha^2+\beta^2)^3} + \frac{4\alpha\beta}{(\alpha^2+\beta^2)^2}t + \frac{\beta}{\alpha^2+\beta^2}t^2\right]. \qquad (30a)$$

(18) As a further case, suppose y given by

$$y = a + bt^3e^{-\alpha t}, \qquad (32)$$

Then it follows that u is given by

$$L(u - u_1) = \frac{be^{-\alpha t}}{a^4}\left[6(2a\alpha^2+g) + 6\alpha(2a\alpha^2+g)t + 3\alpha^2(3a\alpha^2+g)t^2 + \alpha^3(a\alpha^2+g)t^3\right]$$

$$-\frac{be^{-2\alpha t}}{8a^5}\left[9 + 18\alpha t + 18\alpha^2t^2 + 12\alpha^3t^3 + 6\alpha^4t^4 + 12\alpha^5t^5 - 4\alpha^6t^6\right]. \qquad (33)$$

since $a = y_0 = H'$ and u_1 is the final value of u.

Then v is obtained from

$$Bv = Au - bP't^2(3 - \alpha t)e^{-\alpha t}, \qquad (34)$$

and thereafter h is determined from equation (5).

(19) If the acceleration in the surge chamber be omitted, the result in equation (33) becomes

$$L(u - u_1) = \frac{gbe^{-\alpha t}}{a^4}\{6 + 6\alpha t + 3\alpha^2t^2 + \alpha^3t^3\}. \qquad (33a)$$

(20) In general, however, the rate of opening or closing of the gates may be such that the value of y or of v does not follow a simple mathematical law. In such, and, indeed, in all, cases an approximate method of solution of the equations may be adopted, either arithmetical or graphical, in which the frictional terms may also be included.

Moreover, this method may be made to take account of variations in cross-section of the surge chamber when it is not of cylindrical form, *i.e.* when P is not constant, but is a function of the depth y.

Thus if we are given or can assume the curve for y in terms of t we can find the corresponding values of v, u, and h. Or, if we are given a curve showing the variation of v, we can use the method of section (7) to obtain an approximate curve for y in terms of t, and by the following method we obtain the corresponding values of u and h. The curve for y must then be adjusted, if necessary, to secure a closer approximation to the given curve for v.

We divide the whole time of action into a great number of small equal intervals. We then determine from the curve the values of y corresponding to all these values of t, and by taking the difference between consecutive values we obtain the values of $\frac{\delta y}{\delta t}$, either arithmetically in tabular form, or plotted in a curve. In like manner from the series of values for $\frac{\delta y}{\delta t}$ we can obtain

or plot the second differences, corresponding to the values of $\frac{\delta^2 y}{\delta t^2}$. We thus have tabular figures at very short intervals (or plotted curves) for y, $\frac{\delta y}{\delta t}$, and $\frac{\delta^2 y}{\delta t^2}$ from which we can obtain tabular figures (or a further curve) for

$$gy + y\frac{\delta^2 y}{\delta t^2} \pm (g\mu'' y + g\lambda + \tfrac{1}{2})\left(\frac{\delta y}{\delta t}\right)^2, \quad \text{or} \quad Y.$$

Utilising these figures for Y and equation (4) we can determine u as follows:—

At $t = 0$ commence with the known value u_0 of u. As $H - s$ is known in equation (4) and as the term in u^2 is obtained approximately by substituting u_0 for u we find $\frac{\delta u}{\delta t}$. The new value of u then becomes $u_0 + \delta u$, and if we operate in like manner for the second interval of time (substituting the value $u_0 + \delta u$ in the term for u^2) we find the corresponding value of $\frac{\delta u}{\delta t}$.

In this manner step by step the values of u are evolved, either in arithmetical table or plotted in a curve. We have thus obtained the values of u corresponding to the known values of y.

From equation (6) and the tabular values or curve for $\frac{\delta y}{\delta t}$ the values of v may then be deduced.

It is possible then to find the values of h from equation (5), for as the values of Y have been found, and also those of v are determined, the values of $\frac{\delta v}{\delta t}$ may be obtained, and thence h is known. Finally the fluctuation of the power, given by equation (8), may be expressed in tabular figures or by a curve.

This brief description will indicate sufficiently how the approximate method (whether arithmetical or graphical) proceeds.

(21) The arithmetical scheme may be arranged tabularly as in the following example:—Let y be given as a function of t, and let $L = 5000$ feet, $l = 200$ feet, $H = 142$ feet, $s = 100$ feet, $D = d = \frac{4P}{f} = 15$ feet and $A = B = P = 177$ sq. feet, $\lambda = 0$, coefficient of roughness $= \cdot 0003$, so that $g\mu + \frac{1}{2} = 3{\cdot}7$, $g\mu' + \frac{1}{2} = 0{\cdot}625$, $g\lambda + \frac{1}{2} = 0{\cdot}5$, $\mu'' = \cdot 00002$. Let the initial steady velocities be $u_0 = v_0 = 4$ feet per sec. It is required to find the fluctuations of u, v, h, and the water horse-power.

Only the first few columns are shown; for more rapid approximation time intervals of 5 seconds may be taken.

t	0		1		2		3		4		5
δt		1		1		1		1		1	
y	40·15		40·20		40·29		40·45		40·70		41·05
δy		·05		·09		·16		·25		·35	
$\delta^2 y$			·04		·07		·09		·10		
$y\frac{\delta^2 y}{\delta t^2}$			1·608		2·820		3·640		4·070		
gy	1284·8		1286·4		1289·3		1294·4		1302·4		1313·6
$(g\mu''y + g\lambda + \frac{1}{2})\left(\frac{\delta y}{\delta t}\right)^2$		·0013		·0043		·0135		·0329		·0644	
Y	1284·8		1288·0		1292·1		1298·0		1306·5		
$gH' - Y$	59·2		56·0		51·9		46·0		37·5		
u	4		3·9997		3·9987		3·9967		3·9932		
$(g\mu + \frac{1}{2})u^2$	59·2		59·19		59·16		59·10		59·00		
$-L\frac{\delta u}{\delta t}$		1·6		5·23		10·2		17·3			
$-\frac{\delta u}{dt}$		·0003		·001		·002		·0035			
Au	708		707·9		707·8		707·5		706·8		
$P\frac{\delta y}{\delta t}$		8·8		12·4		15·9		17·7			
Bv	708		697·3		693·65		690·7				
v	4		3·940		3·919		3·902				
$-\frac{\delta v}{\delta t}$		·060		·021		·017					
$-l\frac{\delta v}{\delta t}$		12·0		4·2		3·4					
$(g\mu' + \frac{1}{2})v^2$	10		9·702		9·599		9·516				
gh'	1274·8		1286·4		1286·3						
h	139·84		140·20		140·20						
hv	559·4		552·4		549·4						
$\frac{Bhv}{8·83}$	11188		11048		10988						

(22) If the graphical method be followed, it is convenient to arrange the plotting on squared paper; and the work will be rendered rapid and easy by the introduction of certain lines and curves on the sheet.

Plot the given values of y as ordinates to the times t as abscissae. The differences between the consecutive ordinates corresponding to small intervals of time give the corresponding values of δy. These values are plotted as a curve, representing $\frac{\delta y}{\delta t}$. Similarly from this curve the consecutive differences of ordinates are likewise plotted giving the curve for $\frac{\delta^2 y}{\delta t^2}$.

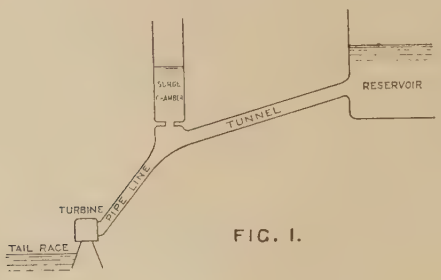


FIG. 1.

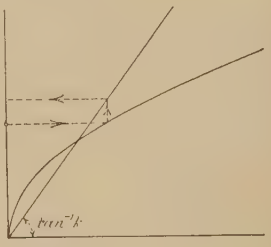


FIG. 2.

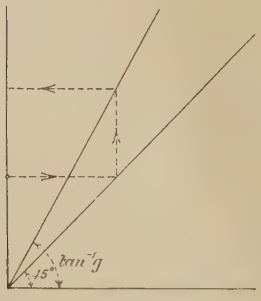


FIG. 3.

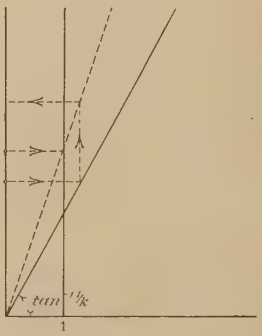


FIG. 4.

Next plot a curve on the diagram giving abscissae equal in length to the squares of the corresponding ordinates (as in fig. 2), and also draw through the origin a straight line making with the horizontal axis an angle whose tangent is K. Then corresponding to any value of $\frac{\delta y}{\delta t}$, K times its square may be at once obtained by following the horizontal line through that value till it meets the square curve, then proceed vertically to intersect the sloping line ($\tan^{-1} K$), and then horizontally to the vertical axis, the intersection of which gives K times the square of the value of $\frac{\delta y}{\delta t}$ that is required.

Further, to multiply a value of y by a constant g it is only necessary to draw a line (fig. 3) through the origin making an angle with the axis of abscissae of which the tangent is g, and another line making an angle of 45°

with the axes. Take the value of y as ordinate, proceed horizontally to intersect the 45° line, then vertically to intersect the line ($\tan^{-1} g$), and finally horizontally to intersect the vertical axis in the value of gy required.

Again to multiply two quantities, both of which are subject to variation such as ky and $\frac{\delta^2 y}{\delta t^2}$, draw an ordinate (fig. 4) distant from the vertical axis by unity on the scale employed for the quantities in question. Draw a straight line through the origin making an angle with the horizontal axis whose tangent is $\frac{1}{k}$. Take on the unity ordinate a height equal to the multiplier $\frac{\delta^2 y}{\delta t^2}$, and through this point draw a straight line from the origin. Take the value of y on the vertical scale and proceed horizontally to meet the sloping line $\left\{\tan^{-1}\frac{1}{k}\right\}$, then vertically to intersect the sloping line just drawn, and then horizontally to the vertical axis where the value of the product of ky and $\frac{\delta^2 y}{\delta t^2}$ required is read. In particular, if $k = 1$, the sloping line $\tan^{-1}\frac{1}{k}$ makes an angle of 45° with the axes.

Or, instead, we may employ an ordinate distant $\frac{1}{k}$ from the vertical axis, and a sloping line through the origin making an angle of 45° with the axes.

For convenience on the diagram different vertical scales may be used for the different quantities involved y, $\frac{\delta y}{\delta t}$, $\frac{\delta^2 y}{\delta t^2}$, etc., coupled with appropriate alteration in slope of the lines drawn through the origin for the purpose of simplification of the multiplication.

By utilising these devices the operation becomes purely mechanical and rapid, and as good an accuracy is obtainable as is warranted by the nature of the data on which the calculations are based. In this way, from the curve for y in terms of the time t, curves for $\frac{\delta y}{\delta t}$, $\frac{\delta^2 y}{\delta t^2}$, Y, $gH' - Y$, u, v, h may be obtained in this order with considerable expedition.

Fig. 5 exhibits some of the results plotted against time as abscissa for the example given in paragraph (21). The various scales for velocity, head, etc., have been so chosen as to bring the several curves into the compass of the diagram.

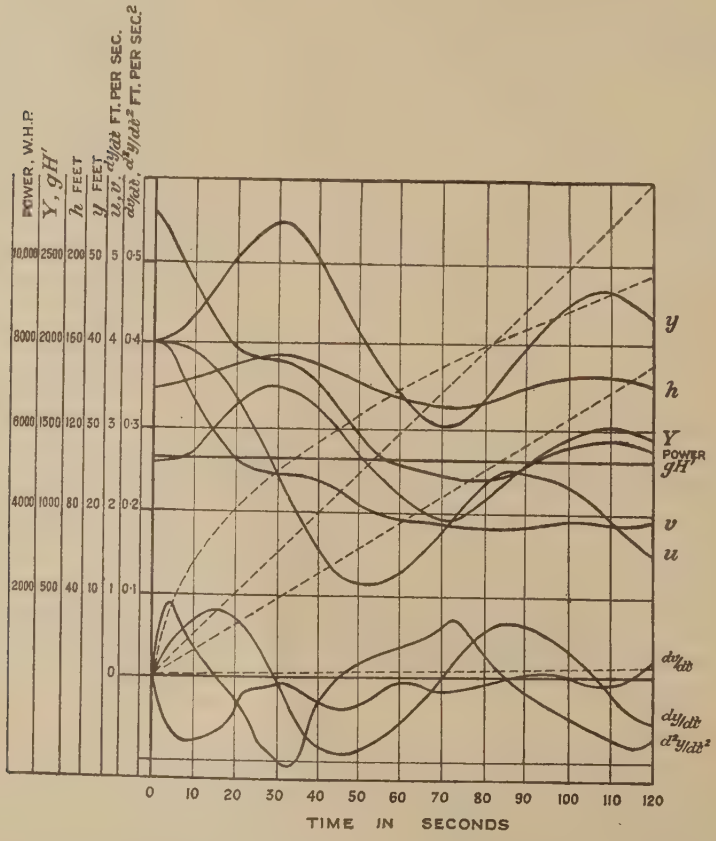
(23) If instead of being given y as a function of t, we were given v (or u) to commence with, we can proceed arithmetically or graphically in a similar manner.

Thus if v is given as a function of t, we use equations (4) and (6) to eliminate u, and then obtain a relation between v,

$$\frac{\delta v}{\delta t} \quad \text{and } y, \quad \frac{\delta y}{\delta t}, \quad \frac{\delta^2 y}{\delta t^2}.$$

As we know v, we can find the values (or curve) of $\frac{\delta v}{\delta t}$; and y must then be chosen to satisfy the equation in y, $\frac{\delta y}{\delta t}$ and $\frac{\delta^2 y}{\delta t^2}$. Commence with the known

initial value of y or y_0 and select the adjacent value $y_0 + \delta y$ so that the differential equation in y is satisfied. Then take the next value of y so that the value of y thus chosen, with its first difference (δy) and second difference ($\delta^2 y$), satisfies the differential equation. And so on, step by step, arithmetically or graphically.



In this case, however, the application of the method is more difficult, as at each step it is necessary to see that both δy and $\delta^2 y$ are chosen so as to satisfy the differential equation, whereas when y is given and u (or v) is sought, the first difference δu is directly determinable.

No. 7.

THE VARIATIONS IN THE QUANTITY OF FOOD REQUIRED BY CATTLE FOR MAINTENANCE AND FAT PRODUCTION WITH DIFFERENT KINDS OF RATIONS.

By JAMES WILSON, M.A., B.Sc.

(Read March 31. Printed April 29, 1925.)

Nearly thirty years ago, when Kellner began the experiments by which he determined the fat-producing capacities of the different food nutrients—starch, fat, protein, and so on—it was essential that the maintenance ration should first be determined with all possible accuracy. Earlier investigators had determined it for several kinds of rations, by varying the food till, judging by the eye and the steelyard, sometimes by the eye alone, they found with what quantity the animal remained constant in weight and condition. Kellner's predecessor, Julius Kühn, had been employing a new method which, it was believed, would give more accurate results, and Kellner continued his work, put together the data collected by both, and interpreted and published the results.[1]

Kühn's method depended upon the assumption that the law of the conservation of energy held in nutrition and that, therefore, none of the energy in the food could be lost. The experimental animals were full-age bullocks, weighing from 11 to 13 cwts., which were fed with such quantities of hay or hay and straw as were expected to maintain them nearly constant in weight and condition. It was known that they could not be fed so that they neither lost nor gained flesh or fat; but small losses or gains could be allowed for.

The bullocks were stalled in a Pettenkofer respiration chamber, by which their solid, liquid, and gaseous intake and outgo could be measured. The caloric values of the food, on the one hand, and of the dung, marsh gas, and urine, on the other, were determined; and the caloric value of the food digested and utilised for maintenance was found by subtracting the latter from the former.[2] The bullock's losses and gains in flesh and fat were calculated from its gains or losses in nitrogen and carbon. If it lost, its ration obviously contained too few utilisable Calories; and the caloric value of the loss added to that of the food actually utilised indicated how many utilisable Calories the bullock required for maintenance. If it gained, the bullock's ration contained too many utilisable Calories; and the utilisable Calories required for maintenance were found by subtracting those required to produce the gain from those actually utilised.[3]

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[1] Untersuchumgen über den Stoff- und Energie- Umsatz des erwachsenen Rindes bei Erhaltungs- und Produktionsfutter. 1900.

[2] *Digested* and *utilised* are nearly equivalent. The constituents in the urine have all been digested, but the energy in a small portion is not utilised.

[3] Kellner assumed that the energy in the flesh or fat—5·678 Calories per gram of flesh and 9·5 per gram of fat—withdrawn from the body was utilised without loss, and calculated that, since 43 per cent. of the food used in his experiments was utilised, 1 Calorie in the flesh or fat was produced by 2·33 Calories in the food.

The following are the essential details of the experiments with six of Kühn's and two of Kellner's bullocks:—

Bullock.	Live weight.	Ration.	In ration.	In excreta.	Utilized.	Flesh gained (+) or lost (−).	Fat gained (+) or lost (−).	Withdrawn from body (+) or supplied in food (−).	Required for maintenance.
	cwts.		Cals.	Cals.	Cals.	gr.	gr.	Cals.	Cals.
II	12·42	18·51 lbs. meadow hay	36975·1	20586·3	16388·8	+ 0·6	+ 86·8	− 1932·3	14456·5[1]
III	12·41	8·51 lbs. clover hay; 8·59 lbs. oat straw	36403·2	19744·2	14859·0	− 15·6	+ 73·5	− 1575·1	13283·9
IV	12·23	8·11 lbs. clover hay; 8·40 lbs. oat straw	33405·1	19265·9	14139·2	− 34·2	− 82·9	+ 981·8	15121·0
V	11·82	17·18 lbs. meadow hay	34211·5	19076·7	15134·8	+ 51·0	+ 126·1	− 3459·6	11675·2
VI	12·65	17·00 lbs. meadow hay	33855·4	17936·8	15918·6	+ 37·8	+ 154·6	− 4147·2	11771·4
XX	13·18	18·72 lbs. meadow hay	37167·3	19726·8	17440·5	+ 19·8	+ 89·0	− 2227·7	15212·8
A	12·17	16·03 lbs. meadow hay	32177·3	15809·0	16368·3	+ 37·2	+ 140·8	− 3601·9	12766·4
B	12·01	7·71 lbs. meadow hay; 9·12 lbs. oat straw	33794·4	18456·7	15337·7	− 87·8	− 105·2	+ 1497·9	16835·6
Average	12·36	(629·5 Kg.)							13890·4
Average, omitting bullock B	12·41	(632·1 Kg.)							13470·0

From these figures, but omitting those for bullock *B*, which did not lie down while its gaseous excretions were being determined, Kellner argued that a 12·4 cwt.[2] bullock required 13,470 utilisable Calories for maintenance; and, since 43[3] per cent. of the food fed in the experiments was utilised, the bullock's whole maintenance ration must consist of as much food as would contain 31,327 Calories (13,470 × 100/43 = 31,327). Since a pound contains about 2,000 Calories, 15·6 lbs. of meadow hay should provide a 12·4 cwt. bullock with all he requires for maintenance.

[1] It may seem a super refinement to give the Calories down to units and decimal places. But the figures are Kellner's, and are given uncurtailed.

[2] The average weight of the experimental bullocks.

[3] This was an under-estimate. It should have been about 45.

It is doubtful, however, if Kellner's argument is sound, for it is not safe to argue from rations containing oat straw, which contains more Calories than meadow hay but requires a greater expenditure of energy for its disintegration and digestion, to rations containing meadow hay alone or to rations containing a mixture of foods still less intractable. We shall see that this is so, if we divide Kühn and Kellner's experiments into three groups and, calculating for each experiment separately as Kellner calculated for all, set down how many Calories and pounds of its own ration each bullock required for maintenance:—

Bullock.	Live weight.	Ration.	Proportion utilised.	Required for Maintenance.			
					Meadow hay.	Clover hay.	Oat straw.
	cwts.		per cent.	cals.	lbs.	lbs.	lbs.
II	12·4	meadow hay	44·3	14456·2	16·28	—	—
V	11·8	do.	44·2	11675·2	13·22	—	—
VI	12·6	do.	47·0	11771·4	12·54	—	—
XX	13·2	do.	46.9	15212·8	16·21	—	—
A	12·2	do.	50·8	12766·4	12·47	—	—
Average	12·44			13176·4	14·14		
III	12·4	clover hay and oat straw	43·0	13283·9	—	7·44	7·50
IV	12·2	clover hay and oat straw	42·0	15121·0	—	8·6	8·95
Average	12·3			14202·5			
B	12·0	meadow hay and oat straw	45·4	16835·6	8·43	—	10·0

Allowing for the restiveness of bullock *B*, it is obvious that, though the proportions of utilisable energy extracted from the different rations were not widely dissimilar, the quantity required for maintenance rose with the addition of oat straw. Even the mixture of clover hay, which is less intractable than meadow hay, and oat straw was more expensive than meadow hay alone.

Kellner's method of determining the fat-producing capacities of the food nutrients and, inferentially, of the foods themselves was a development of that used in these maintenance experiments. A dozen similar bullocks, including *B*, which was used again, were fed for one period with rations slightly over what was necessary for maintenance, but spoken of generally as maintenance rations, which would certainly produce small increases in weight, and the increases determined. The increase consisted usually of both flesh and fat, but the caloric values of the flesh increases were reduced to what they would have been had they been increases in fat, and the whole increases expressed as in fat. The bullocks were then fed during other periods with the same maintenance rations plus measured additions of starch meal, gluten meal, earthnut oil, and other substances, and the further increases in weight determined. The fat produced by the added substances was found by subtracting that produced by the maintenance ration from that produced by the maintenance ration plus each added substance. For example, bullock *C* was fed

for three periods—the first being the maintenance period—and made the following increases :—

Period.	Live weight.	Food.					Increases.		
		Meadow hay.	Dried beet.	Rye bran.	Starch meal.	Gluten meal.	Flesh.	Fat.	Total as fat
	cwts.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
i	11·86	10·34	7·73	1·91	3·54	0·59	0·211	1·61	1·73
ii	12·83	10·52	7·70	1·91	7·05	0·59	0·128	1·91	2·06
iii	13·11	10·74	7·75	1·92	3·55	3·95	0·268	2·10	2·26

Neglecting the slight differences in the quantities consumed—they were meant to be alike—it is obvious (subtracting the figures in the first experiment from those in the others) that 3·51 lbs. of starch meal produced 0·33 lb. of fat and 3·36 lbs. of gluten meal 0·53 lb. of fat. Therefore, when added to the maintenance ration, 10·64 lbs. of starch meal or 6·36 lbs. of gluten meal were required to produce 1 lb. of fat.

Kellner did not wish to know, however, how many pounds of total starch or gluten meal, but how many pounds of digestible starch or gluten meal were required to produce a pound of fat. By other experiments he had found that 2·16 of the 3·51 lbs. of starch and 2·576 of the 3·36 lbs. of gluten meal were digestible, and he might have argued that, since 2·16 lbs. of digestible starch or 2·576 lbs. of digestible gluten produce a pound of fat, therefore 6·55 lbs. of digestible starch or 4·86 lbs. of digestible gluten are required, when added to a maintenance ration, to produce 1 lb. of fat. He argued in another way, however.[1] He drew up a table showing how many Calories were in each of the bullock's rations, how many in the excreta, how many utilisable, how many were required for maintenance, *on the assumption that the bullock required no more when putting on fat than when idle,* and how many were left over for fat production. The table is as follows :—

Period.	Live weight.	In whole ration.	In excreta.	Utilisable.	Required for maintenance.	Left over for production.
	cwts.	Cals.	Cals.	Cals.	Cals.	Cals.
i	11·86	47458·0	20669·1	26788·8	13067·5[2]	13721·4
ii	12·83	54016·5	24187·3	29929·9	13772·0	16157·2
iii	13·11	56293·6	23209·0	33086·4	13972·3	19112·3

Then, converting the Calories in the last three columns back into food, he calculated how much of the food in each ration had been utilised, how much required for maintenance, and how much left over to produce fat; and subtracting the quantity of utilisable food left over in experiment i from the quantities in experiments ii and iii, and subtracting the quantity of fat produced in experiment i from the quantities produced in experiments

[1] Here his argument is shortened and simplified.

[2] These figures were calculated by Meeh's rule,

ii and iii, he found the surplus utilisable food consumed and the surplus fat produced in experiments ii and iii as follows:—

Period.	Utilisable food.	Required for maintenance.	Left over for production.	Surplus.	Fat produced.	Surplus.
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
i	16·348	8·217	8·131	—	1·731	—
ii	18·170	8·659	9·510	1·379	2·057	0·326
iii	19·115	8·789	10·322	2·181	2·257	0·526

From this, he argued that, since 1·379 lbs. of utilisable or digestible starch added to a maintenance ration produce ·326 lb. of fat and 2·181 lbs. of digestible gluten produce ·526 lb. of fat, therefore, to produce 1 lb. of fat, 4·2 lbs. of digestible starch or 4·15 of digestible gluten (i.e. protein) must be added to the maintenance ration.

But is this method sound? The difference between the proportions of total to digestible starch or protein required to put on a pound of fat, when maintenance is neglected and when it is not, namely, 10·64 : 4·2 for starch and 6·36 : 4·15 for protein, suggest that something may be wrong. To indicate what may be wrong, let us construct an assumed but not impossible example. If, in the third period of the experiment with bullock *C*, 10·74 lbs. of oat straw were substituted for the 10·74 lbs. of meadow hay, the whole ration would contain about 58,000 Calories, the excreta only a little more, if any, than 23,200, and about 35,000 would be utilisable. If the bullock required no more for maintenance, as Kellner assumed, than when idle, namely, 13,972·3 Calories, then about 21,000 Calories would be left over for fat production. But the bullock, with 21,000 Calories left over for production, would put on less fat on this ration than on the experimental one with only 19,000 Calories left over for production. Let us assume he puts on as much fat with the straw ration as with the experimental one. Then the food utilisable, required for maintenance, and left over for production, with the surpluses in utilisable food consumed and fat produced, would be as follows:—

Period.	Utilisable food.	Required for maintenance.	Left over for production.	Surplus.	Fat produced.	Surplus.
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Hay, etc.	16·348	8·217	8·131	—	1·731	—
Straw, etc.	19·800	8·217	11·583	3·452	2·257	0·526

Thus, about 6·56 lbs. (3·452/·526) of protein instead of 4·15 would have to be added to the maintenance ration to produce 1 lb. of fat.

Kellner made the working assumption that the bullock requires no more for maintenance when putting on fat than when idle, and the unqualified assumption that the bullock requires no more for maintenance with a less than with a more tractable ration. When a bullock is idle, the whole of the energy in his food is spent in keeping the organism repaired and working, in disintegrating, manipulating, and digesting the food, and in maintaining the body temperature. When putting on fat, he consumes more food, and more energy is spent in its disintegration, manipulation, and digestion; and, though no more may be spent in keeping the body warm, more may be spent in repairing the organism and keeping it working.[1] Moreover, some portion

[1] All the extra energy, whether used in digestion, conversion of food, or otherwise in maintenance, finally leaves the bullock as heat.

of the food is retained in the body, but not in its original form, and more energy must be spent in converting this portion into its new forms and transporting and depositing them in their proper places. The following diagram represents what happens :—

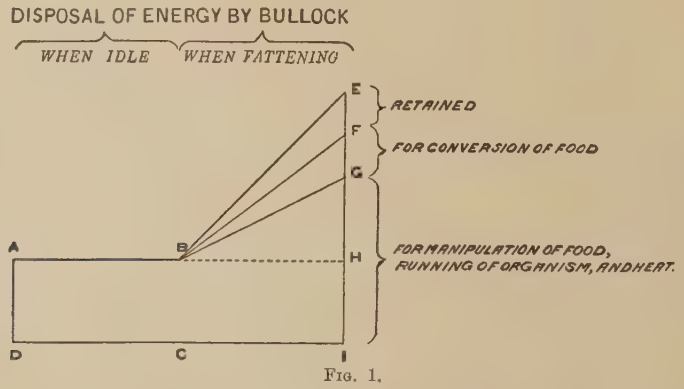


Fig. 1.

If the line *EI* represent the digestible or utilisable energy in the ration and *EF* that retained by the animal, Kellner assumed that the line *HI* represents the energy required for maintenance, but omitted from maintenance the energy represented by *FH*; whereas, since his experimental bullocks were doing nothing but living and putting on fat, all the energy not retained by them must have been used for maintenance, and should be represented by the line *FI*. Thus, since he underestimated the utilisable energy required for maintenance and, therefore, overestimated the utilisable energy left over for fat production, his estimates of the quantities of the digested nutrients required to produce a pound of fat must be too high. If maintenance be underestimated, more will seem to be left over for production and more to be required to produce a pound of fat. Nor can they be even relatively accurate; for, since the lines *FG* and *GH* vary with the amount of fat put on and the tractability of the ration, his estimates must also vary with the lengths of these lines. They are applicable relatively only to rations similar to, and producing at the same rate as, those from which they were obtained.

The following table contains the essential data provided by Kühn and Kellner's experiments—maintenance and subsequent—to indicate what is necessary for maintenance and production at different rates of production. Maintenance now means all the utilisable energy in the ration excepting that retained by the animal. Where necessary, to distinguish between the energy required by the animal when idle and when producing, we shall call the former idle utilisable maintenance and the latter producing utilisable maintenance.[1]

From this table it is obvious and to be expected that the Calories in the whole ration and, though, perhaps, less to be expected, that the producing utilisable Calories generally rise with the fat put on by the bullock. The rations consumed by the first eight bullocks, putting on from none to a third

[1] Armsby calls the whole utilisable energy in the ration—that retained plus that required for maintenance—the metabolisable energy.

of a pound a day, contained, on an average, 34,523·7 Calories, of which, on the average, 15,116·4 were required for producing utilisable maintenance, while the rations consumed by the next seven bullocks, putting on nearly two pounds a day, contained, on an average, 52,604·9 Calories, of which, on an average, 20,088·5 were required for producing utilisable maintenance. The extra energy required for disintegrating, manipulating, and digesting the food and for converting some of it into fat amounted to nearly 5,000 Calories per bullock per day.

The table also shows that the Calories required for producing utilisable maintenance vary not only with the fat put on by the bullock but also with the ingredients of the ration, especially with the kind and quantity of the long fodder. It is generally assumed that a bullock must have long fodder. Till concentrates were introduced, the bullock which was not working had long fodder only in winter, and to-day the bullock which is not being fattened has frequently long fodder only. But, though he may be "stored" on long fodder only, he cannot be fattened, at any rate, fattened quickly, without something more concentrated. And, since his stomach is limited in capacity, and if more fat is to be produced, more food and more Calories must be consumed, it is obvious, since they produce more, bulk for bulk, than long fodder, that the concentrates must be increased and the long fodder reduced. It is almost equally obvious, from experience with ourselves and animals—a man does not overload his own or his horse's stomach if either is to do something requiring severe exertion—that the bullock's ration is likely to decline in efficiency, as his stomach is taxed beyond a certain proportion of its capacity: which is likely to be well short of the maximum. Thus, since its bulk is greater than an equal weight of concentrates, it is likely that long fodder begins to overtax the stomach sooner than concentrates.

[See Tables on following pages.]

Bullock.	Live weight.	Period.	Hay.	Straw.	Dried beet slices.	Rye bran.	Starch.	Fibre.	Gluten.	Earthnut oil.	Earthnut meal.
	cwts.		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
V	11·82	—	17·12	—	—	—	—	—	—	—	—
B	12·01	—	7·7	9·12	—	—	—	—	—	—	—
A	12·17	—	15·98	—	—	—	—	—	—	—	—
IV	12·23	—	8·08	8·36	—	—	—	—	—	—	—
III	12·41	—	8·48	8·56	—	—	—	—	—	—	—
II	12·42	—	18·44	—	—	—	—	—	—	—	—
VI	12·65	—	16·95	—	—	—	—	—	—	—	—
XX	13·18	—	18·66	—	—	—	—	—	—	—	—
B	11·94	i	9·71	—	7·76	1·94	3·62	—	3·97	—	—
	12·5	ii	9·62	—	7·76	1·91	7·02	—	·6	—	—
	12·99	iii	9·63	—	7·68	1·92	3·55	—	3·96	—	—
	13·22	iv	9·68	—	7·70	1·92	3·56	—	·59	—	—
C	11·86	i	10·57	—	7·76	1·92	3·55	—	·59	—	—
	12·83	ii	10·49	—	7·73	1·91	7·03	—	·59	—	—
	13·11	iii	10·7	—	7·77	1·92	3·56	—	3·97	—	—
III	12·57	ii	8·27	8·51	—	—	3·66	—	—	—	—
	12·24	iii	8·11	8·4	—	—	3·68	—	1·29	—	—
	13·66	iv	8·38	8·44	—	—	3·70	—	2·59	—	—
IV	12·39	ii	8·34	8·41	—	—	3·55	—	—	—	—
	12·42	iii	8·59	8·52	—	—	3·56	—	1·3	—	—
V	12·17	2a	17·05	—	—	—	3·59	—	—	—	—
	12·57	2b	17·10	—	—	—	3·68	—	—	—	—
VI	13·26	2b	17·16	—	—	—	3·69	—	—	—	—
	13·59	iii	17·10	—	—	—	6·17	—	—	—	—
D	13·44	i	13·01	—	4·78	5·77	—	—	—	—	—
	14·33	ii	13·33	—	4·77	5·75	3·51	—	—	—	—
	14·73	iii	13·30	—	4·77	5·75	—	—	—	1·59	—
	15·67	iv	13·54	—	4·81	5·77	—	—	3·2	—	—

[1] Drawn from t

In whole ration.	In excreta.	Utilisable.	Retained.				Expended in maintenance.	Proportion of whole ration retained.
				Flesh.	Fat.	Calculated as fat.		
Cals.	Cals.	Cals.	Cals.	gr.	gr.	lbs.	Cals.	per cent.
34211·5	19076·7	15134·8	1487·6	·51	126·1	·34	13647·2	4·35
33794·4	18456·7	15337·7 + 1497·9[1]	—	—	—	—	16835·6	0·
32177·3	15809·0	16368·3	1548·8	37·2	140·8	·36	14819·5	4·81
33405·1	19265·9	14139·2 + 981·8[1]	—	—	—	—	15121·0	0
34603·2	19744·2	14859·0 + 88·6[1]	715·4	—	75·3	·17	14232·2	2·07
36975·1	20586·3	16388·8	830·9	·6	86·8	·19	15557·9	2·25
33855·4	17936·8	15918·6	1683·3	37·8	154·6	·39	14235·3	4·97
37167·3	19726·8	17440·5	957·9	19·8	89·	·23	16482·6	2·58
54939·3	21483·5	33455·8	8338·8	126·6	802·1	1·93	25117·0	15·18
54928·6	20984·7	31943·9	8314·8	43·4	849·3	1·93	23629·1	15·11
54469·0	20253·1	34215·9	8758·1	134·7	841·4	2·03	25457·8	16·08
46129·1	17468·7	28660·4	5846·2	64·9	576·6	1·35	22814·2	12·67
47458·0	20669·1	26788·9	7477·9	96 1	729·7	1·73	19311·0	15·73
54016·5	24087·3	29929·2	8886·4	58·4	900·5	2·06	21042·8	16·45
56293·6	23209·0	33084·6	9748·5	121·9	953·3	2·26	23336·1	17·32
40964·5	21298·8	19665·7	3006·7	58·2	281·7	·67	16659·0	7·34
44025·3	21654·1	22371·2	4076·7	85·2	378·2	·94	18294·5	9·26
48293·6	23226·3	25067·3	5287·3	48·6	527·2	1·22	19780·0	10·95
40725·6	21935·1	18790·5	1761·6	40·2	161·4	·43	17028·9	4·32
44860·3	22162·3	22698·0	4010·1	53·4	390·2	·93	18687·9	8·94
40827·5	20339·2	20488·3	4268·5	84·	399·1	·99	16219·8	10·45
40917·4	20706·0	20211·4	4014·2	24·	408·2	·93	16197·2	9·81
41245·9	20094·4	21151·5	4062·3	73·8	383·5	·94	17089·2	9·85
45859·6	21510·2	24349·4	5413·8	99·	510·7	1·25	18935·6	11·79
46945·4	20987·2	25958·2	6616·6	76·6	650·7	1·53	19341·6	14·10
53902·2	23308·4	30593·8	8769·3	82·3	873·9	2·03	21824·5	16·27
54007·3	22622·2	31385·1	8931·0	85·	889·3	2·07	22454·1	18·02
56053·6	23824·7	32228·9	8221·9	111·2	799·	1·90	24007·9	14·67

nimal's reserves.

Bullock.	Live weight.	Period.	Hay.	Straw.	Dried beet slices.	Rye bran.	Starch.	Fibre.	Gluten.	Earthnut o	Earthnut
	cwts.		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs
E	13·24	i	13·01	—	4·76	5·76	—	—	—	—	—
	14·14	ii	13·52	—	4·78	1·92	3·49	—	—	—	—
	14·67	iii	13·30	—	4·78	1·92	—	—	—	1·59	—
	15·08	iv	13·47	—	4·83	1·93	—	—	3·2	—	—
F	11·45	i	14·34	—	3·83	3·83	—	—	·4	—	—
	12·09	ii	7·65	7·62	3·81	3·81	—	—	·4	—	—
	11·72	iii	7·58	—	3·84	3·83	—	—	·4	—	—
	12·13	iv	7·68	—	3·85	3·83	3·74	—	·4	—	—
	12·78	v	7·71	—	3·85	3·82	—	—	·4	1·76	—
	12·90	vi	6·96	—	3·84	3·82	—	—	·4	—	—
G	12·14	i	7·55	7·47	7·68	—	—	—	·5	—	—
	12·39	ii	14·11	—	7·66	—	—	—	·5	—	—
	12·22	iii	7·63	—	7·62	—	—	—	·5	—	—
	12·66	iv	7·72	—	7·68	—	3·71	—	·5	—	—
	12·88	v	7·47	—	7·67	—	—	—	·5	1·76	—
H	12·69	i	7·52	7·53	5·68	—	—	—	—	—	1·98
	12·95	ii	14·92	—	5·63	—	—	—	—	—	1·95
	13·04	iii	7·44	—	5·65	—	4·46	—	—	—	1·94
	13·14	iv	7·56	—	5·65	—	—	—	—	—	1·93
	13·73	v	7·69	—	5·63	—	—	6·03	—	—	1·95
	13·97	vi	7·71	—	5·70	—	—	—	—	—	1·96
	14·45	vii	15·35	—	5·71	—	—	—	—	—	1·97
J	12·08	i	7·51	7·51	5·64	—	—	—	—	—	1·94
	12·34	ii	14·98	—	5·63	—	—	—	—	—	1·92
	12·32	iii	7·57	—	5·63	—	3·54	—	—	—	1·93
	12·47	iv	7·66	—	5·65	—	—	—	—	—	1·94
	13·03	v	7·69	—	5·67	—	—	6·03	—	—	1·94
	13·34	vi	7·74	—	5·68	—	—	—	—	—	1·95

[1] Drawn from t

In whole ration.	In excreta.	Utilisable.	Retained.				Expended in maintenance.	Proportion of whole ration retained.
				Flesh.	Fat.	Calculated as fat.		
Cals.	Cals.	Cals.	Cals.	gr.	gr.	lbs.	Cals.	per cent.
46888·8	20492·5	26396·3	5917·7	104·6	560·4	1·37	20478·6	12·6
46240·2	20913·4	25326·8	4336·8	95·7	399·3	1·	20990·	9·38
45997·8	19333·0	26664·8	5923·1	103·2	561·8	1·37	20741·7	12·88
47890·1	21187·2	26702·9	3145·9	84·4	280·7	·73	23557·	6·57
44821·2	21414·5	23406·7	4592·6	209·9	352·	1·06	18814·1	10·25
46690·1	23273·3	23416·8	4229·6	118·5	374·4	·98	19187·2	9·06
31327·8	13647·9	17679·9	2058·7	32·8	196·5	·48	15621·2	6·57
38608·3	15614·5	22993·8	5388·	158·2	472·6	1·25	17605·8	13·96
39185·9	17261·5	21924·4	4487·5	119·4	401·	1·05	17436·9	11·45
37946·2	15382·9	22563·3	4627·7	134·4	406·8	1·08	17935·6	12·20
45626·1	22912·7	22713·4	3683·4	122·5	314·5	·85	19030·	8·07
43811·3	20552·9	23258·4	3938·3	106·	351·2	·92	19320·1	8·99
30751·8	13376·	17375·8	1777·3 + ·2[1]	0·	187·1	·41	15598·3	5·78
37963·6	14935·0	23028·6	5367·2	102·5	503·7	1·24	17661·4	14·14
38057·3	16282·2	21775·1	4672·1	43·5	465·8	1·08	17103·0	12·28
45570·1	23595·4	21974·	2736·7	26·9	272·	·63	19237·3	6·00
45255·8	19809·7	25446·1	6258·3	142·5	573·6	1·45	19187·8	13·87
38562·4	14519·6	24042·8	5808·7	77·7	565·	1·34	18234·1	16·23
30338·1	12895·4	17442·7	2057·1	43·4	190·6	·48	15385·6	6·78
41900·7	15492·5	26408·2	7873·	157·	734·9	1·82	18535·2	18·79
37544·4	14441·4	23103·0	6378·8	98·8	612·4	1·48	16724·2	16·99
46275·	20142·3	26132·7	5762·9	96·4	549·	1·33	20369·8	7·71
45365·9	22735·7	22630·2	3565·8	54·3	342·9	·83	19064·4	7·86
45239·6	19404·2	25835·4	5205·8	152·4	456·9	1·32	20629·6	11·51
36982·6	14362·2	22620·4	4995·8	90·3	471·9	1·16	17624·6	13·09
30548·5	12677·1	17871·4	2301·5	32·9	222·6	·53	15569·9	7·53
41962·6	15530·1	26432·5	7139·7	98·3	692·8	1·65	19292·8	5·51
37461·1	14525·1	22936·	4561·2	91·4	425·5	·94	18374·8	12·18

[1] nimal's reserves.

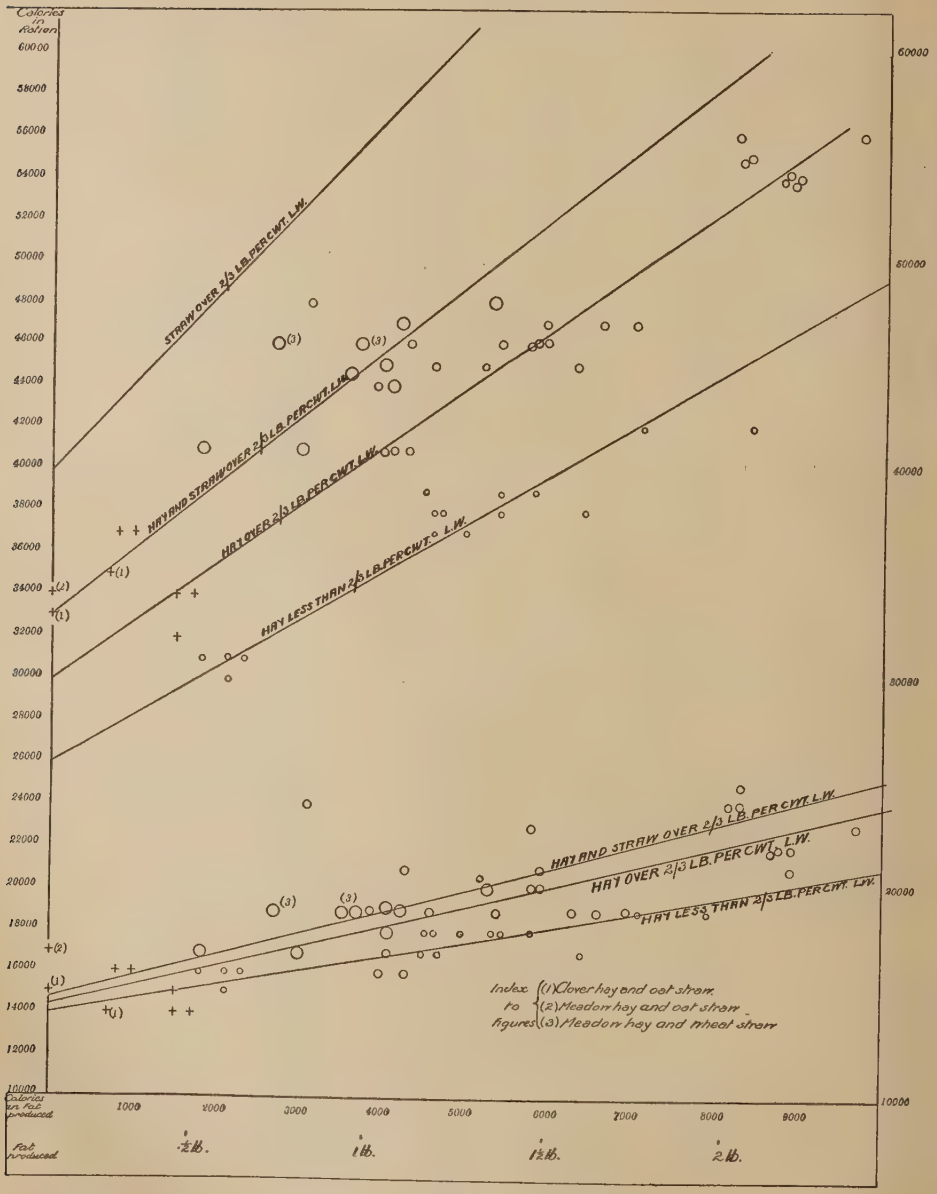

Calories in ration
60000
58000
56000
54000
52000
50000
48000
46000
44000
42000
40000
38000
36000
34000
32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
STRAW OVER 2/3 LB. PER CWT. L.W.
HAY AND STRAW OVER 2/3 LB. PER CWT. L.W.
HAY OVER 2/3 LB. PER CWT. L.W.
HAY LESS THAN 2/3 LB. PER CWT. L.W.
HAY AND STRAW OVER 2/3 LB. PER CWT. L.W.
HAY OVER 2/3 LB. PER CWT. L.W.
HAY LESS THAN 2/3 LB. PER CWT. L.W.
Index figures (1) Clover hay and oat straw
(2) Meadow hay and oat straw
(3) Meadow hay and wheat straw
Calories in fat produced
1000
2000
3000
4000
5000
6000
7000
8000
9000
Fat produced
½ lb.
1 lb.
1½ lb.
2 lb.

FIG. 2.

A casual examination of Kellner's rations and their results shows that a mixture of hay and straw makes a ration less efficient than an equal weight of hay alone; and a closer examination that the rations containing a larger quantity of hay are less efficient than those containing a smaller. The rations have, therefore, been divided according to their long fodder content, into four groups, and both the total Calories in each and those required for producing utilisable maintenance plotted, in the opposite diagram, against the Calories in the flesh and fat each produced. The plotting marks for the whole rations and for their utilisable portions—the former in the upper group, the latter in the lower—are, for rations containing long fodder alone, a cross; for those containing concentrates and less than two-thirds of a pound of hay for every live hundredweight of the animal, a very small circle; for those containing concentrates and over two-thirds of a pound of hay for every live hundredweight, a medium circle; and for those containing concentrates and hay and straw, a large circle. Through the average of both groups of plottings for the three kinds of rations containing concentrates lines have been drawn, from which can be read off how many Calories must be contained in a 12·4 cwt. bullock's whole ration and how many are required for producing utilisable maintenance, when the bullock is fed with different kinds of rations and putting on fat at different rates. Another line is drawn higher up across the diagram which may be taken as a guess at how many Calories should be in the bullock's ration, were the long fodder to consist of 13 to 17 lbs. of oat straw.

From this diagram it is obvious that the food—total and digestible—required by the bullock, whether idle or fattening, rises with the quantity and kind of long fodder in the ration, and rises also with the rate at which fat is being put on by the bullock. For every pound of fat put on, the digestible or producing utilisable Calories rise by 3,000, 4,000, and 4,500, when the ration contains less than two-thirds of a pound of hay, more than two-thirds of a pound of hay, and more than two-thirds of a pound of hay and straw, per live hundredweight; while the Calories in the whole ration rise by 10,000, 12,000, and 13,500 with the same three variations in long fodder. These results are expressed, for the whole ration, in the following table, which shows how many Calories, how many pounds of meadow hay equivalent (a pound of meadow hay contains, on the average, 2,000 Calories), and how many pounds of barley equivalent or fodder units (a pound of barley is equivalent, on the average, to $2\frac{1}{2}$ lbs. of meadow hay) are required by a 12·4 cwt. bullock when idle and when putting on fat at different rates with these three kinds of rations and, presumably, with a ration in which the long fodder consists of straw alone:—

Fat put on	0			1 lb.			2 lbs.			3 lbs.		
	Calories.	Hay equivalent : lbs.	Fodder units.	Calories.	Hay equivalent : lbs.	Fodder units.	Calories.	Hay equivalent : lbs.	Fodder units.	Calories	Hay equivalent : lbs.	Fodder units.
with concentrates and 2/3 lb. of meadow hay per cwt. live weight.	26000	13·0	5·2	36000	18·00	7·2	46000	23	9·2	56000	28·00	11·2
with concentrates and over 2/3[1] lb. meadow hay per cwt. live weight.	30000	15·0	6·0	42000	21·00	8·4	54000	27	10·8	66000	33·00	13·2
with concentrates and over 2/3[1] lb. hay and straw per cwt. live weight.	33000	16·5	6·6	46500	23·25	9·3	60000	30	12·0	73500	36·75	14·7
with concentrates and a similar quantity of straw.	40000	20·0	8·0	58000	28·00	11·2	72000	36	14·4	—	—	—

Reduced to the rations required by cattle of ordinary weights, by Meeh's rule, $\frac{\text{food required by A}}{\text{food required by B}} = \frac{3/2\sqrt{\text{live weight of A}}}{3/2\sqrt{\text{live weight of B}}}$, and expressed in fodder units, these figures become—

Live weight of bullock,	Required for idle or simple maintenance. 6 cwts.	7 cwts.	8 cwts.	9 cwts.	10 cwts.	11 cwts.	Required in addition for production.
	Fodder units.	Fodder units.	Fodder units.	Fodder units.	Fodder units.	Fodder units.	
with concentrates and less than 2/3 lb. meadow hay per cwt. live weight.	3·15	3·46	3·8	4·09	4·38	4·64	2 fodder units per pound of fat put on.
with concentrates and more than 2/3[1] lb. meadow hay per cwt. live weight.	3·6	3·99	4·36	4·72	5·05	5·36	2·4 fodder units per pound of fat put on.
with concentrates and over 2/3[1] lb. of hay and straw per cwt. live weight.	3·97	4·39	4·8	5·19	5·56	5·89	2·7 fodder units per pound of fat put on.
with concentrates and over 2/3[1] lb. of straw per cwt. live weight.	4·78	5·32	5·8	6·29	6·73	7·14	3·2 fodder units per pound of fat put on.

[1] In Kellner's experiments the quantity ran from about $\frac{2}{3}$ to $1\frac{1}{3}$ lb. per live hundredweight.

To those unaccustomed to question Kellner's results, these figures may seem astounding. Kellner found that the fat-producing capacities of the food nutrients varied with their caloric value: that a pound of fat produced about 2¼ times as much fat as a pound of starch. Thus, if a pound of food fat were to produce a pound of body fat, 2¼ lbs. of starch would be required to produce a pound of body fat. But, in the experiments in which the rations contained less than two-thirds of a pound of hay per hundredweight live weight, a pound of body fat was produced by every two pounds of the ration over and above that required for gross utilisable maintenance. The only fat in most of the rations was that in the hay, which contains only about ½ per cent. digestible. Thus, if the protein in the rations be regarded as less fat-productive than carbohydrates, according to their caloric value, the carbohydrates in these rations produced more fat than they were entitled to do. Is it that the nutrients do not produce fat according to their caloric value, or that the experiments are too few, or that the lowest line through the upper group of plottings is wrongly drawn?

Kellner's estimate that 4 pounds of digestible starch added to a maintenance ration produce a pound of fat fails with the pig, when its ration does not contain too much fibre. With rations containing less than about 5 per cent. of fibre, pigs weighing not more than about 1¾ cwt. not infrequently put on a pound of bacon for every 3½ pounds of meals fed. It is usually assumed that about a third of the ration of a pig of this size is required for maintenance. Then, if maintenance be allowed for, the pig puts on a pound of bacon, most of which is fat, with about 2·33 lbs. (3·5—1·17) of meals. If the pig's maintenance requirements are calculated as Kellner calculated the bullock's, a third of its food for maintenance is too low, and maintenance allowed for, it probably puts on a pound of bacon with less than 2·33 lbs. of meals. And if this can be done by the pig, why not also by the bullock, if fed with equally tractable rations? The bullock is much larger and better covered, and should need a relatively smaller proportion of its food to maintain its body temperature.

In a long series of fattening experiments made by British farmers, a digest of which, by Mr. Herbert Ingle, is to be found in the 1909 volume of the "Transactions" of the Highland and Agricultural Society of Scotland, many of the experimental animals, fed with more than two-thirds of a pound of hay per day per live hundredweight, some with hay and straw, and most with more roots than Professor Nils Hansson has shown to be the optimum,[1] put on a pound of beef—maintenance being allowed for—with less than four fodder units, i.e. less than 4 lbs. of barley equivalent, and less than 3 lbs. of starch equivalent, a day.

The results of Kellner's experiments attract attention to an inference of great economic importance which cannot be mentioned too often, namely, that the more tractable it is and the quicker it produces, the more economical the ration. The last column in the tables on pp. 85 and 87 shows how much of each whole ration was retained by the animal and, by inference, how much lost. If this column be scanned it will be seen that most was retained and least lost with the more tractable rations which were fed in quantity sufficient to produce the largest gains.

The writer's attention was re-drawn to the depressing effect of large quantities of long fodder in cattle rations, by those fed during the closing period of the great war. Since that time he has discussed the subject frequently with his former pupil, Mr. E. J. Sheehy, of the animal nutrition section of the Irish Free State Department of Agriculture, whose criticisms and suggestions have contributed materially to what is in this paper.

[1] Rotfruktsgivornas Optimum belyst genom Utfodringsförsök med Mjölkkor, Stockholm, 1924.

No. 8.

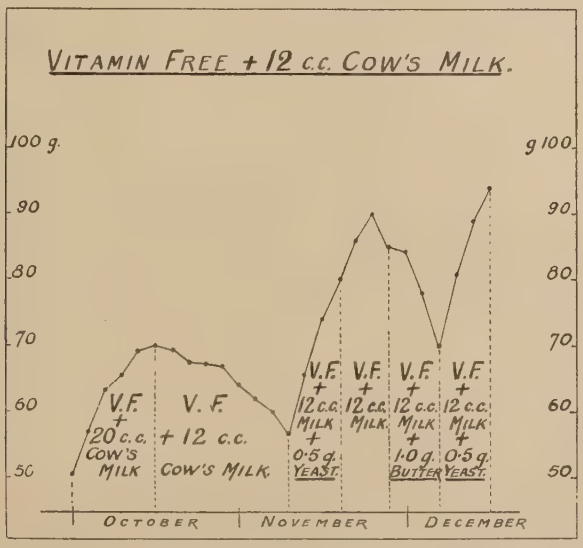
THE IDENTITY OF VITAMIN A. THE COMPARATIVE EFFECTS OF HUMAN AND COW'S MILK.

By HAROLD PRINGLE,
Physiological Department, Trinity College, Dublin.

[Read March 31. Printed April 27, 1925.]

Introduction.

However varied the natural sources of the different vitamins employed in nutrition experiments, it has been generally assumed that each represents an individual rather than a class. The effects of deprivation and administration are such as suggest that these accessory factors are definite and distinct chemical entities.



AVERAGE OF 3 YOUNG RATS, 2 MALES, 1 FEMALE. WEIGHTS at 3 DAY INTERVALS

Curve I.

This does not appear to have been seriously questioned in the cases of Vitamin B and Vitamin C, but there are grounds for speculation as to the individuality of Vitamin A.

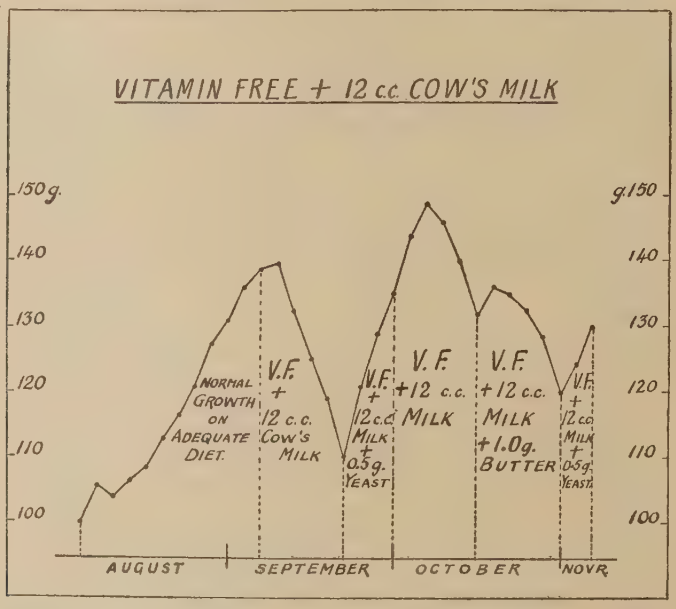
The problem may be stated as follows:—Is the growth-promoting vitamin always the same substance however varied the source of supply? Is the growth

process, however it may differ in different species, regulated by a factor common to all species?

In approaching this problem a distinction must be drawn between the quantitative variations which are known to occur in the vitamin present in the same source of supply and the qualitative variations, which are the subject of this paper.

Quantitative Variations in Vitamin Contents of Natural Sources.

It is to be expected that a given natural source of vitamin will always contain the same vitamin, although the proportions may vary with varying conditions of production. This has been shown to be true for cow's milk, the vitamin content of which varies considerably with the diet of the animal (Drummond, Coward, and Watson, 1921; Kennedy and Dutcher, 1922), the amount of milk fat secreted (Drummond, Coward, Golding, Mackintosh, and



AVERAGE OF 3 ADULT RATS, 2 MALES, 1 FEMALE. WEIGHTS AT 3-DAY INTERVALS.

CURVE II.

Zilva, 1923), and the influence of sunlight (Hume, 1923; Luce, 1924). A similar kind of variation is probable in any other natural source of Vitamin A, such as cod-liver oil. The recognition of these quantitative variations is of the utmost importance in feeding experiments.

Qualitative Variations in the Vitamin A Content of Different Natural Sources.

On purely theoretical grounds it may be doubted if the normal growth vitamin is the same for the tissues of a rapidly growing species, e.g. the rat, as for the tissues of a slowly growing species such as the tortoise.

As a preliminary simplification the growth process may be divided into two components, (1) the organised factor determined by the species, and (2) the unorganised factor supplied by the vitamin. Vitamins may operate in one of at least two ways, as reactants and as catalysts. As reactants, they enter into cell-metabolism as structural components of living tissues, and only differ from the recognised biochemical compounds in the low concentration demanded by the cells. As catalysts, they modify the growth-process without contributing energy to it. The evidence in support of each of these possible modes of action will not be discussed here, as there are not sufficient data available to provide a decisive conclusion. However, it appears unlikely that both types of function can be served by one class of substances.

If Vitamin A is to be considered as a reactant in a cell-system, it is probable that it is a single definite chemical individual, however varied its source. If it be a catalyst, its individuality is not so obvious, as the growth-catalysts may well vary with the different growth-substrates. A series of investigations as to the influence of the growth-vitamin from two biologically distinct sources, human and cow's milk, acting on a common growth-substrate, the young rat, was undertaken to see if any evidence could be obtained as to a qualitative difference in vitamin from two different sources.

Experimental.

Young albino rats under the usual conditions of experiment (Drummond, 1921) were employed. The vitamin-free diet consisted of:

Glucose, pure	25 parts.
Casein, pure, B.D.H.	10 „
Fat, Jurgen's Vitamin free Margarin	10 „
Salt Mixture	4 „

The composition of the salt mixture was as follows:—

Potassium Carbonate	141 grams.
Calcium Carbonate	134 „
Citric Acid	111·1 „
Phosphoric Acid	103 „
Hydrochloric Acid	53 „
Sodium Carbonate	34 „
Magnesium Carbonate	24 „
Sulphuric Acid	9·2 „
Ferric Citrate	6·34 „
Sodium Chloride	0·248 „
Manganese Sulphate	0·079 „
Potassium Alum	0·024 „
Potassium Iodide	0·02 „

In each case where an extra article of diet was added, milk, yeast, or butter, the rat was taken from its cage previous to feeding, and watched till it had consumed the quantity given.

The cow's milk was an average sample of Dublin milk obtained from a local dairy.

CURVE I.—Average of three young rats, two males and one female.

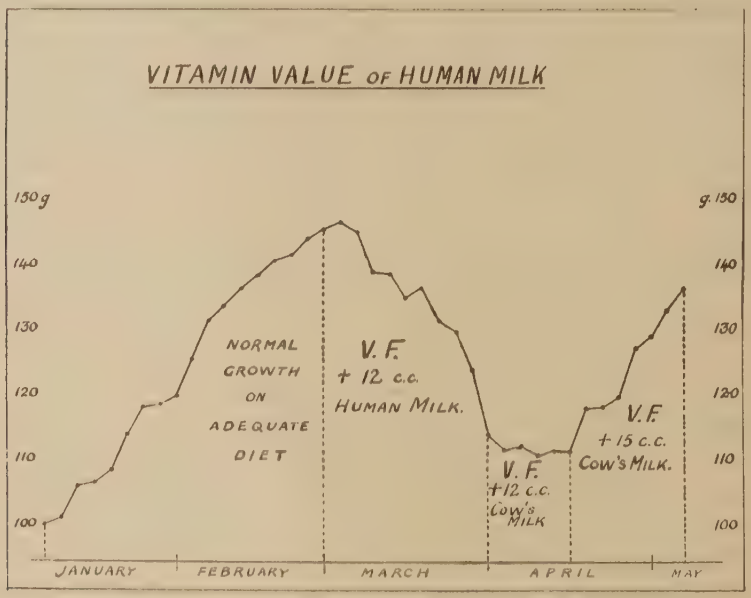
This curve shows that young rats maintain a normal growth on 20 c.c. cow's milk daily, but lose on 12 c.c. daily. That this is probably due to

deficiency of Vitamin B is shown by the marked improvement on giving a small quantity of yeast daily, while the addition of butter has no effect.

Curve II.—Average of three adult rats, two males one female.
Similar to Curve I, except that adult rats were used instead of young rats.

Curves I and II show low vitamin content of Dublin milk.

Curve III shows the vitamin value of human milk, 12 c.c. of which per diem were not sufficient to maintain weight, which fell rapidly. 12 c.c. cow's milk replacing the human milk checked the fall in weight and maintained equilibrium, while addition of 15 c.c. cow's milk caused an increase in weight and a return to the previous maximum value.



AVERAGE OF 2 RATS. WEIGHTS AT 3 DAY INTERVALS.
Curve III.

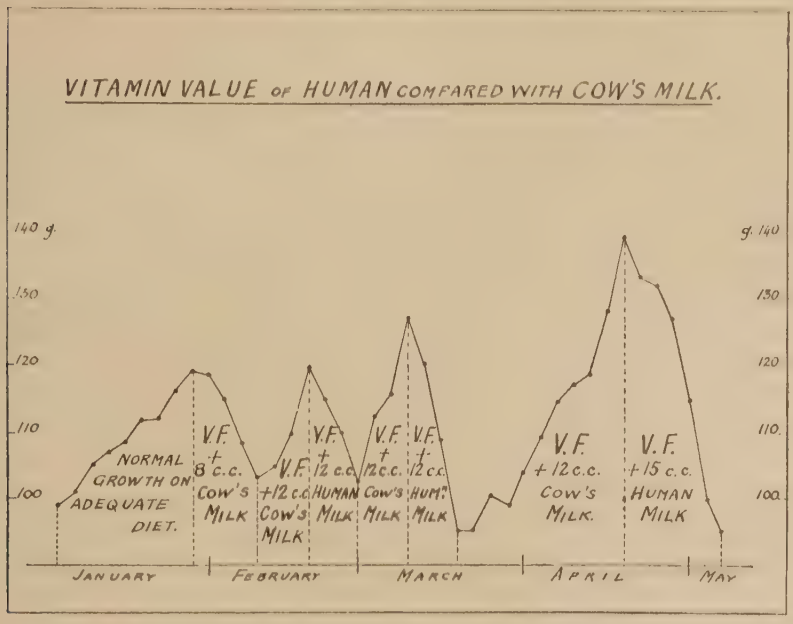
Curve IV shows the instantaneous loss in weight each time 12 c.c. cow's milk was replaced by human milk. This curve is the most interesting of the series, since it appears to indicate a fundamental difference between human and cow's milk as regards growth factors.

Under normal circumstances, when a rapidly growing animal is suddenly deprived of its growth vitamin or has its supply reduced below a critical value, its weight becomes stationary for a variable period, and then declines. The duration of the stationary period depends on the supply of vitamin stored in the tissues of the animal, and, in turn, is determined chiefly by the quantity of fat present. The administration of human milk in each case resulted in a sharp drop in weight without any previous stationary period, which suggests that human milk contains either a growth-retarding factor for the

tissues of a more rapidly growing animal than man, or else Vitamin A is one of a class of catalysts which accelerates growths according to the requirements of the particular species, or else human milk is deficient in Vitamin B. The rapidity of the weight fall does not support this.

In no case was growth obtained on vitamin-free diet with human milk. Further experiments with increasing amounts of human milk are in progress.

In the classical experiments of Hopkins, 2 c.c. of milk were sufficient to maintain normal growth in young rats. Osborne and Mendel, working in Melbourne, found 10 c.c. per day necessary.



AVERAGE OF 2 RATS. WEIGHTS AT 3 DAY INTERVALS

CURVE IV.

In the above experiments Curves I and II show that 12 c.c. per day were insufficient, whereas Curve IV shows 12 c.c. promoting normal growth. This difference is probably due to seasonal variation in the quality of the milk. As a result of numerous experiments the minimum for Dublin dairy milk was found to vary from 9–12 c.c.

I wish to acknowledge my indebtedness to Dr. Cassidy, Master of the Coombe Hospital, Dublin, for enabling me to obtain supplies of human milk.

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No. 9.

ON THE PHOTO-ELECTRIC MEASUREMENT OF SUBMARINE ILLUMINATION.

By H. H. POOLE.

[Read April 28. Printed May 28, 1925.]

Introduction.

It is evident that the use of a photo-electric cell has much to recommend it, on the score of convenience, for the purpose of routine measurements on the illumination at moderate depths below the surface of the sea. Shelford and Gail[1] have employed a cell of the Kunz pattern for such measurements in the waters of Puget Sound. Shortly after the publication of their paper Dr. W. R. G. Atkins suggested to me that the carrying out of similar measurements in the English Channel would be most useful in connection with the work on the seasonal variations of plankton which is in progress at the Marine Biological Association's station at Plymouth. An account is given below of the apparatus which has been constructed for this purpose, and of some preliminary measurements which have been carried out, in conjunction with Dr. Atkins, at Cawsand Bay, near Plymouth. The results obtained were of such a promising nature that it is hoped that, in the near future, numerous measurements of light penetration will be made under varying conditions.

Measurement of Photo-Electric Current.

A study of Shelford and Gail's paper showed that these workers had the advantage of fine weather and water so smooth that a galvanometer could be employed as a measuring instrument in a ten-ton vessel. As these conditions would seldom occur in the English Channel, the first problem was to devise a method of current measurement dispensing with the use of any instrument requiring a steady support.

The principle adopted is as follows:—The photo-electric current is passed through a high resistance R (see fig. 1), and the P.D. between the ends of the latter is balanced against a potentiometer P. A telephone is employed as a detector, an interrupter I being included in the telephone circuit, which is thus made and broken 100 or more times per second. It is obvious that, if the potentials are not accurately balanced, we may expect to hear a more or less musical sound in the telephone. It was found, however, that the currents to be measured were so small that this simple arrangement did not give a fine enough balance. Accordingly a two-stage valve amplifier A was introduced to magnify the telephone currents. Further considerable enhancement of sensitivity was effected by the introduction of a condenser C, connected as shown in the figure. This charges up during the intervals when the interrupter has broken the amplifier primary circuit, and discharges through the latter when contact is made again.

[1] Publications, Puget Sound Biological Station, III, 65, December 20, 1922, p. 141.

For the sake of simplicity only the main circuits are shown in the figure, which represents the general scheme of the connections. The high-tension battery *B* is of the usual type used for wireless reception capable of giving various potentials up to 120 volts in 6 to 8 volt steps. By the aid of an auxiliary small battery of 6 cells, of the type used for obtaining a grid bias, any pressure up to nearly 130 volts can be obtained to within less than a volt. The actual pressures of the various combinations used are tested daily with a high resistance voltmeter. All the connections to these batteries are made with wander plugs, as used in wireless work. These proved very convenient in enabling connections to be changed quickly.

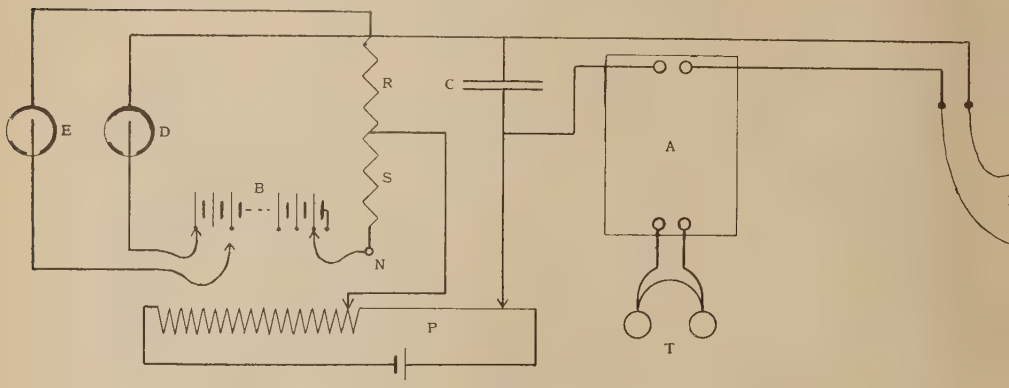


Fig. 1.

D and *E* represent photo-electric cells. Two types of cell are used, one being a Kunz cell of the type in which the sensitivity is increased by ionisation by collision, and the other a vacuum cell made at the General Electric Company's research laboratory. As it was necessary to take comparative readings of both cells at the briefest possible intervals their negative leads are permanently connected to the resistance *R*, while their positive leads are fitted with wander plugs which can be quickly plugged in to the appropriate socket in the battery. This action requires care, as if the Kunz cell is inadvertently connected to too high a potential while it is exposed to bright light its sensitivity is temporarily altered.

The resistances *R* and *S* are of the "Zenite" wire type, *R* being 51,300 ohms, and the safety resistance *S* being about 19,000 ohms. The potentiometer, shown diagrammatically at *P*, is of the type made by Messrs. Tinsley for hydrogen ion concentration measurements. In its normal setting it reads directly to 10^{-3} volt, but can be shunted so that each division represents 10^{-4} volt. On this range, which was the one employed, 513 potentiometer divisions correspond to one microampere. As it was found that a setting could be made to within about 0·5 scale division we may take it that the arrangement enables currents to be measured to within 10^{-9} ampere. The potentiometer current is supplied by a small accumulator cell [Exide type D.T.G.]. The condenser *C* is of one microfarad capacity, and is mica insulated. A somewhat smaller one works equally well, but good insulation is essential.

The amplifier *A* is a two-valve transformer-coupled Marconi note magnifier, fitted with Marconi Osram D.E.V. valves, the filament current being supplied

by two dry cells. The most suitable plate voltage seems to lie between 15 and 30 volts, and is supplied by a small dry battery. A step-up transformer is used between the input terminals and the grid of the first valve, and a step-down transformer between the plate circuit of the second valve and the telephones. Thus the filament and H.T. batteries of the amplifier are insulated from both the potentiometer and the telephone circuits.

Considerable difficulty was experienced in making an interrupter, shown diagrammatically at *I*, which would not introduce extraneous E.M.F.'s into the circuit. The form finally adopted was made out of an electric bell, which was converted into a buzzer by replacing the vibrating armature by one much more rigidly mounted, so as to increase the frequency of oscillation. To this is attached a light vulcanite arm about 3 inches long, carrying at its end a small platinum fork, to which is attached a fine [40 S.W.G.] copper wire leading to a fixed bind screw forming one terminal of the interrupter. The prongs of this fork are a few millimetres long and about 0·5 mm. apart. They are mounted in a plane perpendicular to the axis of the arm, the prongs being perpendicular to the direction of the to-and-fro motion, which takes place in their plane. From the second terminal a similar copper wire leads to a point on the vibrating arm about 1·5 cm. from the fork, and is there soldered to a small brass bolt passing through the vulcanite. From this anchorage it passes on, and carries at its free end a short length of fine platinum wire. The copper wire between the anchorage and the platinum is bent into an S form, and so adjusted that the platinum wire normally passes between the prongs of the little forks, without bearing heavily on either of them. It is evident that when the buzzer is in action the circuit is made when the platinum wire is thrown against either prong of the fork, and is interrupted twice per complete oscillation. A 0·4 microfarad condenser, connected in parallel with the buzzer contact breaker, greatly improves the working, and helps to reduce the extraneous E.M.F.'s which the buzzer is liable to generate in neighbouring circuits, and which make the attainment of a silent balance impossible.

In order to render this effect unimportant it was found necessary to make the buzzer local circuit as compact as possible, and to keep it at least 5 feet away from the rest of the apparatus, connection being made from the interrupter to the amplifier circuit by means of twin flexible leads. The buzzer, its dry cell, switch, and condenser were accordingly mounted in a wooden box forming a separate unit. The handle of the rotary switch passes through the side of the box, so that the buzzer can be readily started or stopped as required. The wires and contacts forming the interrupter are situated in one corner of the box, and screened from the buzzer by a sheet of copper. To reduce the sound of the buzzer it is carried on cork pads.

When carefully adjusted and placed at a sufficient distance from the other circuits this interrupter works well. The sound heard in the telephone may generally be described as "scratchy." This is not much disadvantage, as telephones are usually very sensitive to such sounds, although a pure musical note would probably be easier to hear amid sea sounds. The sensitivity can to a large extent be regulated by adjusting the filament current of the amplifier valves. If this is too small the sensitivity is insufficient, while if it is too large silence is unattainable, but when it is suitably adjusted the sensitivity is amply sufficient, even with considerable noise due to waves.

All the apparatus, except the photo-electric cells and connecting leads, the interrupter, and the head telephones, is mounted in a stout wooden box about 2 feet square by 1 foot deep. This renders the arrangement comparatively portable, so that it can be used almost anywhere and can be very quickly

put into operation. Great care is necessary with the insulation as the apparatus may have to work under very damp conditions. The H.T. batteries are mounted on paraffin wax blocks, and all the rest of the insulation is of vulcanite. The leads to the submarine photometer are of the best quality high-tension rubber-insulated cable, as used for ignition, the over-all diameter being 11 mm. Those to the deck photometer are of a lighter type of rubber-insulated cable, being about 5 mm. over-all diameter. Guard ring wires are wound round the two positive cables, not far from their terminals, and connected to the negative bind-screw *N*. These wires, which are not indicated in the figure, prevent currents leaking along the surface of the rubber-insulation from affecting the readings. Before these were fitted considerable currents, say 50 scale divisions or 10^{-7} ampere, might be observed in very damp conditions with the photo-electric cells screened from light. These currents, which may for convenience be referred to as "dark currents," although in no case did they appear to pass through either of the actual photometer cells, were greatly reduced by the guard wires, and only became troublesome when the air was so damp that it caused a visible film of moisture on the ebonite surfaces. Under these conditions a dark current might either be positive or negative according to circumstances. A positive dark current can be measured and allowed for, if it is sufficiently steady, but a negative one cannot be read without reversing the potentiometer conditions, which is inconvenient. It was found that the dark current could be altered by connecting the negative bind-screw *N* (see fig. 1) to one of the three points on the two-cell filament battery. Connection to the negative terminal produces a positive dark current and vice versa. This artifice may be adopted if it is necessary to make observations under very bad conditions, but the results so found will never be as reliable as those obtained under good conditions, when the dark currents are sensibly zero, and *N* is not connected to the filament battery. The vacuum cell is fitted with both internal and external guard rings, which are connected to *N* by a special lead not shown in the figure. The Kunz cell, used in the submarine photometer, has an external guard ring of tinfoil connected to the metal photometer case.

Photo-electric Cells.

Two cells of the Kunz type were obtained from the Central Scientific Co., Chicago. One of these, denoted henceforth by the letter *K*, has been used throughout in the submarine photometer, while the second has been reserved for any laboratory tests that may become desirable during the progress of the work. The colour sensitiveness of Kunz cells has been studied by Shinomiya[1] and Miss Seiler,[2] who have shown that the maximum sensitivity, while depending somewhat on the particular alkali metal employed, is in the neighbourhood of 4,500 A.U., i.e., in the blue part of the visible spectrum.

Preliminary tests with artificial light showed that over the available range, 0–25,000 metre candles, the current was very approximately proportional to the illumination. As, however, the maximum obtainable in these tests fell far short of full sunlight, considerable doubt would be felt in extra-polating from these results. Moreover, it soon appeared that the cell was anything but a satisfactory standard. If too large a current be passed through the cell the immediate result is to increase the sensitivity considerably. This effect is transient, and by next day the cell has usually attained approximately its original sensitivity. It is evident, then, that when the cell is to be exposed to bright

[1] Astrophys. Jour., *49*, p. 303, June, 1919.
[2] „ „ *52*, p. 129, Oct., 1920.

light the applied voltage must be reduced in order to keep the current within safe limits. After considerable experience the rule was eventually adopted that, with the cell mounted in the submarine photometer, as described below, the applied pressure is to be as nearly as possible 20 volts for illuminations exceeding 8,000 m.c., and may be increased to 120 volts below that limit, this increase raising the sensitivity about fourteen times. In this way the current is limited to about 3 microamperes, corresponding to a potentiometer reading 1550×10^{-4} volt.

In spite of this precaution, however, the constancy of the cell could not be relied on, so it was proposed to standardise the cell daily by means of a specially constructed electric lantern. This is not a very satisfactory solution, as the lantern is somewhat cumbrous, and involves a considerable increase in the amount of apparatus required. Moreover, it is only capable of giving illuminations up to about 25,000 m.c.

Just at this time a letter appeared in *Nature* (April 26th, 1924) from the Research Staff of the General Electric Co. announcing the construction of a vacuum photo-electric cell, which, though much less sensitive than the Kunz type, is stated to be absolutely constant and to give a current accurately proportional to the illumination. Through the courtesy of the Director of the G. E. C. research laboratory, a potassium cell of this type, denoted *G* hereafter, was obtained and used as a standard, for which purpose it appears to be ideal. It was mounted in the deck photometer, and used both as a standard of comparison for the submarine photometer and as a means of observing the variations in the surface light, which is seldom constant.

Submarine Photometer.

Figure 2 shows, on a scale of 1/2, how the *K*-cell is mounted in the strong gun-metal case used to protect it from the hydrostatic pressure. It will be seen that the cell is attached to the lid by vulcanite clips and metal screws with rubber buffers interposed below it so that it is held in a flexible, yet positive, manner almost, but not quite, in contact with the lower surface of a plate-glass window 1 cm. thick. The inner surface of this window is obscured with the matt varnish used for muffing electric lamps. A black card diaphragm is held in contact with this surface by the edge of the sheet-rubber gasket which forms the water-tight joint between the window and the metal. The aperture in this diaphragm is 2·4 cm. in diameter, so the photometer actually measures the illumination on a horizontal diffusing surface 4·5 sq. cm. in area, forming the lower surface of a sheet of glass 1 cm. thick. It was estimated that, with the photometer in air, the mean semi-vertical angle of the cone of unobstructed illumination is about 80°, neglecting the obstruction caused by the cables and the screw glands. The angle in water would be somewhat less, but would considerably exceed the critical angle, so that no appreciable obstruction would occur unless a cable, or one of the screw glands, happened to cast a direct shadow on to the window. This can be prevented, at least at moderate depths.

The method of making the joints round the cables is shown in the figure. The cable passes through a brass screw plunger and through a rubber stopper, the rubber insulation butting against an ebonite bushing, through which the copper core passes on into the vessel. On screwing up the plunger the rubber stopper, which may be lubricated with a little vaseline, is compressed and a satisfactory joint is made. A shoulder prevents the ebonite bushing from being forced into the vessel. The joint between the lid and the bottom of the photometer case is fitted with a rubber gasket and secured by twelve $\frac{3}{8}''$

gun-metal bolts and nuts, those at the corners being eye-bolts, to which the supporting wire rope bridle is shackled. This bridle extends about 1·1 metres above the photometer and terminates in a thimble which is attached by a swivel clip-hook to the warp which supports the apparatus. The cables are attached to two of the bridle wires in such a way that, even if the former are hanging quite slack, sharp bends are prevented.

The leads extend to a considerable depth in the vessel, so that in the event of a leak they would be short-circuited by the rising water before the pressure rose sufficiently to break the cell. The trouble would thus be discovered and the photometer immediately hauled up. In the bottom of the vessel is placed a little calcium chloride wrapped up in a felt pad, which is held in place by a couple of ebonite clips. This keeps the air in the vessel dry. Owing to the length of the glass seal at the end of the cell remote from the anode, which

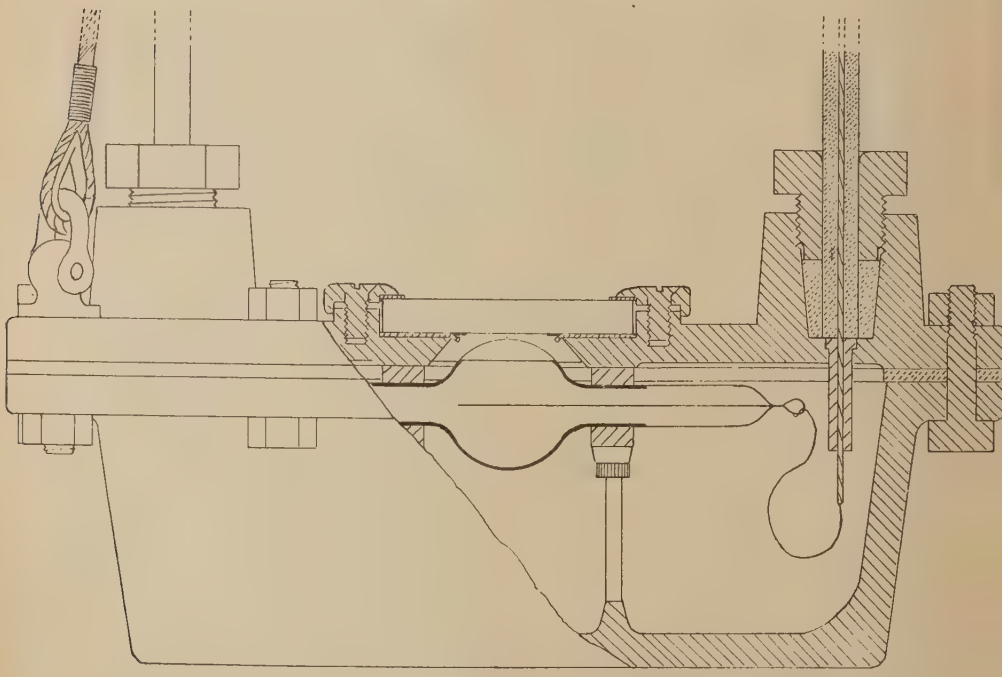


Fig. 2.

is shown in the figure, it was necessary to mount the cell with its long axis making a small horizontal angle with the axis of the vessel, so that the seal might clear the ebonite bushing at the negative end. This inclination, which is not indicated in the figure, does not, of course, affect the working in any way. A strip of tinfoil gummed round the anode of the cell is electrically connected to the case.

An external brass shutter, large enough to cover the window and its encircling brass rim, and provided with a lip to prevent the entry of any light, is attached by a ball-joint to an arm connected to an axle which works

in bearings parallel to the long edge of the photometer case. By rotating this axle the shutter can be made to close the window completely, or can be removed and dropped below the level of the window, so that it cannot throw any shade on the latter. The weight of the shutter is partially supported, when the photometer is submerged, by a cork float, so that it can be easily operated by means of strings.

Deck Photometer.

As the *G*-cell is much smaller than the *K*-cell it is fitted in a wooden box so that its "pupil," which forms a hemisphere about 1 cm. in diameter projecting from the bulb, is just below the inner surface of a thin glass window. In the preliminary experiments described below this window had a ground lower surface, the effective aperture being about 1 cm. in diameter. This was chosen as it was felt that matt varnish might reduce the light too much with this somewhat insensitive cell. As, however, the diffusing properties of the ground surface seemed to be insufficient, the window was subsequently replaced by a larger one with matt varnish on its lower surface, and an aperture 2·4 cm. in diameter mounted in a sheet-metal photometer lid in such a way that light is received unobstructed from almost an entire hemisphere.

Initial Standardisation of Deck Photometer.

As the cells measure chiefly the blue light, the sensitivity in visual metre candles will evidently depend on the colour of the light used as a standard. Quite different results were obtained with a carbon arc and a metal filament lamp. The former, though a most unsatisfactory source, was used as approximating more nearly to daylight in colour. The current was maintained as nearly as possible at 10·0 amperes by hand regulation, the voltage across the arc being about 53·5. Its visual candle-power was found by comparison with a 16 c.p. standard carbon filament lamp working at 4·05 watts per candle, a flicker photometer being employed. The mean of five measurements gave the candle-power of the arc as 1250, the extremes recorded being 1070 and 1380. Nine readings of the photo-electric current were made with the arc at 12·2 cm. from the photometer window. The variations were similar in magnitude to those observed visually, the mean potentiometer reading being 428 with an anode voltage of 119·3. As the variation of sensitivity with anode voltage is only about 0·3 per cent. per volt in the neighbourhood of 120 volts, this correction is almost inappreciable. Taking it into account we find that 1 scale division, $1{\cdot}95 \times 10^{-9}$ ampere, corresponded to 195 metre candles, i.e. about 100,000 m.c. would produce one microampere. With the cell so mounted, perpendicular sunlight caused a reading of about 800 scale divisions corresponding to 156,000 metre candles. The fact that this exceeds the usual estimate of sunlight is, of course, attributable to the greater percentage of blue light in sunlight than in the arc light with which the photometer was standardised. As the chief interest of the measurements lies in the comparison of the surface with the submarine illumination the actual scale employed is not of very great importance.

Optical Conditions affecting the Measurements.

We must consider carefully what the illumination recorded by one of the photometers really represents. As it is obviously necessary to protect the diffusing surface from the action of the water it must be placed on the inner surface of the window. Thus the photometer may be assumed to measure the total light falling on a horizontal diffusing surface of definite area forming

the posterior surface of a sheet of glass. Now we actually wish to measure the illumination at a point immediately in front of the photometer window. We must, accordingly, multiply the photometer reading by a factor, in order to correct for the reflection losses at the front surface of the glass, and also for the effect of obliquity. It is evident that different factors will be required for air and water, and that in either case the value will depend on the average angle of incidence of the light.

We must first define what we mean by the illumination at a given point, choosing our definition in such a way that the quantity arrived at may be a true measure of the photo-synthetic activity of the light. The problem is complicated by the fact that in the case of daylight we have light of varying intensity coming from all possible angles. It seems probable that the photo-synthetic activity of the light is almost independent of the angle, whereas the illumination on any plane surface obviously depends on the orientation of the surface relative to the light, and no single surface can be perpendicular to all the light.

We may, in our definition, neglect all light travelling in an upward direction, as it does not directly affect either photometer, and so is not measured. There is obviously a great deal of this light above the surface of the sea, but it cannot contribute directly to the submarine illumination. Beneath the surface there can be relatively little light passing upwards.

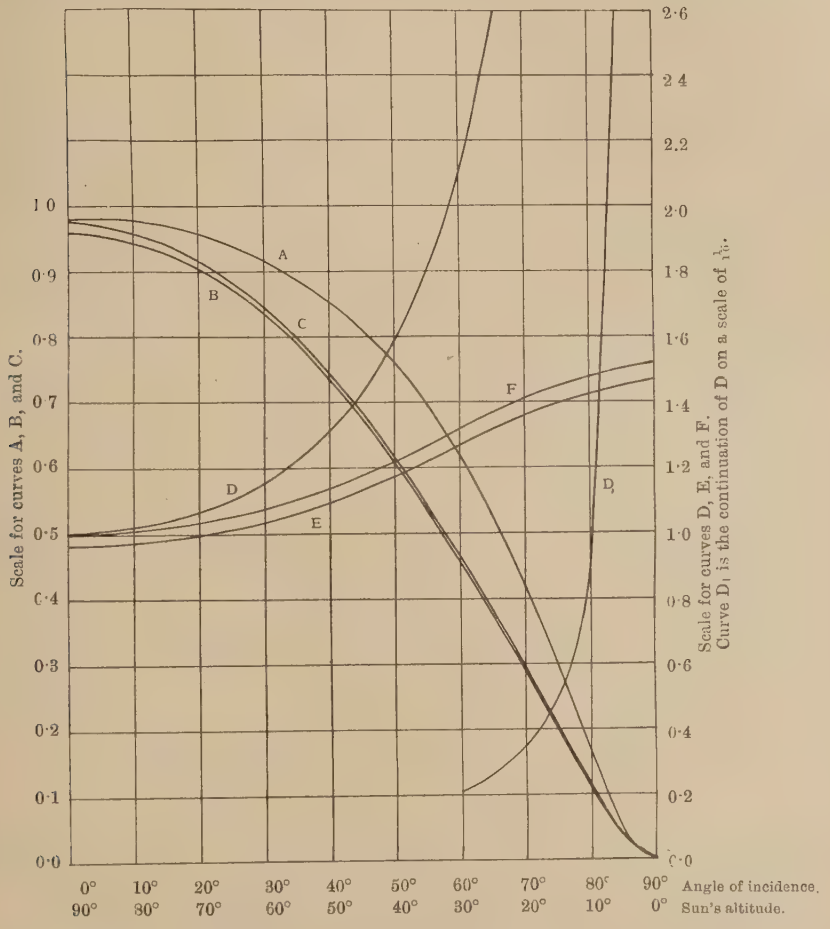
We will accordingly define the illumination as follows:—Let $id\Omega$ be the illumination in metre candles due to a given small pencil of light of solid angle $d\Omega$ on a small surface at the given point perpendicular to the axis of the pencil. Then the total illumination I is defined as $\int_0^{2\pi} id\Omega$. We thus include in our definition all the light coming from the upper hemisphere regardless of angle.

The actual angular distribution of this light will evidently be very variable. It is probable, however, that we will seldom make a serious error if we assume that a certain proportion of the light at a point in air is direct sunlight in a definite direction, determined by the position of the sun, while the remainder is perfectly diffused light from a sky equally bright all over. For direct sunlight our definition of illumination evidently becomes simply the illumination in metre candles on a small area perpendicular to the light. For perfectly diffused light i is constant over one hemisphere, so $I = 2\pi i$. This is easily seen to be twice the illumination on a horizontal surface at the point.

Considering first direct sunlight, we can, by employing Fresuel's formulae, plot a curve, *A* (fig. 3), showing the relative illumination at a point just below a smooth water surface (refractive index 1·333) for various angles of incidence. Assuming the refractive index of the glass to be 1·51, and allowing for the obliquity of the ray in the glass to the photometer surface, we can, in a similar way, plot other curves showing, *B*, the relative light flux on a photometer window in air, and, *C*, the same for a photometer just immersed in water. It will be noticed that, owing to the action of the water in reducing the reflection losses, the submerged photometer reads slightly higher than when dry. This was borne out by experiment. Tests made with the deck photometer (*G*-cell) illuminated with artificial light (a Mazda "Daylight" gas-filled lamp) at various angles agreed well with curve *B*.

From these curves we can obtain the required factors for direct light. Since the standard (deck) photometer was calibrated by means of direct light at perpendicular incidence, no correction being made for reflection losses,

it is evident that the value of the air factor D must be unity for these conditions. The corresponding value of B is 0·96, the perpendicular reflection loss being 4 per cent. Thus the value of D for any other angle of incidence is evidently 0·96 divided by the value of B for that incidence. The values so obtained are plotted, D_1 representing the continuation of the curve on $\frac{1}{10}$th scale. The



"Diffuse" values of factors, A = 0·56, B = 0·45, C = 0·47 D = 2·12, E = 1·14.

FIG. 3.

water factor E must depend on the ratio of the illumination in water, A, to the light reaching the immersed photometer, C. As before the coefficient 0·96 must be introduced, since, although the submarine photometer was not directly calibrated at perpendicular incidence, it is daily compared, under identical

conditions, with the deck photometer, which was so standardised. Thus $E = \frac{0{\cdot}96\,A}{C}$. For small angles of incidence E < 1, since there is less reflection loss at the water—glass surface than occurred during the calibration at the air—glass surface. E is always much less than D since the light is more nearly vertical in water and, as there is also much less reflection loss, is more efficient in affecting the photometer. Thus a given reading implies less actual light when the photometer is submerged than when it is above the surface.

For diffused light we divide the sky into nine zones, the extension of each in altitude being 10°. Taking the values of A, B, and C for the mean altitude of each zone, and multiplying each by the solid angle subtended by the zone, we find, on dividing the sums of the products by 2π, the values of A, B, and C for diffused light. From these the corresponding values of D and E are obtained as before. These "diffuse" factors for the deck photometer are shown below the figure. In the case of the submarine photometer, when used in air, the screening off of the lowest 10° zone by the brass rim encircling the window causes a reduction of about 1·3 per cent. in the "diffuse" value of B, and a corresponding increase in that of D. When the photometer is submerged this error vanishes since there cannot, under water, be much light making an angle with the vertical greater than the critical angle. A small correction, depending on the proportion of diffuse to direct light, can easily be applied to the air standardisation of the submarine photometer to allow for this effect.

It is also necessary to know the angle of refraction (r) of the light in water, since, as pointed out by Shelford and Gail, the optical depth at a given point is proportional to its secant, which is accordingly plotted (curve F) in the figure. For diffused light we must adopt some mean value of F. Now as the penetrating power, and hence the importance, of the light increases with decrease of F, i.e. with increase of $\cos r$, it seems to be more natural to find the weighted mean value of $\cos r$, i.e. $\frac{1}{F}$, rather than that of F. We accordingly proceed as follows:—If the solid angle subtended by one of the zones be Ω the illumination due to it just below the surface will be proportional to $A\Omega$. Then evidently the mean value of

$$\frac{1}{F} = \frac{\Sigma \frac{A\Omega}{F}}{\Sigma A\Omega} \quad \text{i.e. diffuse value of } F = \frac{2\pi \times \text{diffuse value of } A}{\Sigma \frac{A\Omega}{F}}.$$

This method, though not strictly logical, since the effect of the light is not directly proportional to $\cos r$, is probably close enough for practical purposes.

The effect of depth on the average obliquity of illumination is complicated. Absorption would evidently increase the relative importance of vertical rays, so that its effect would render the illumination at considerable depths almost wholly vertical. Scattering will, on the other hand, have the opposite effect. In the absence of information as to the relative importance of these two factors we may, for the present, assume that they roughly balance one another, so that the average angle of obliquity is not much affected by the depth. We accordingly use the same obliquity factor, and the same value of $\sec r$ for all depths.

Preliminary Experiments near Plymouth.

Some preliminary tests were carried out in conjunction with Dr. Atkins at Cawsand Bay, near Plymouth, in the early autumn of 1924. As the time available for these tests was very limited, and much of it was wasted by bad weather, little more was done than to gain some experience of the behaviour of the apparatus. One set of tests was carried out on board the steam-drifter "Salpa," belonging to the Marine Biological Association, when moored, in about 10 metres of water, opposite to the old torpedo station at Cawsand Bay, the rest of the observations being made on the pier attached to the station, the submarine photometer being lowered from a small crane so that it was about 10 feet clear of the pier, which is an iron and timber structure. Measurements were made down to the full depth of water available according to the state of the tide. It was found that, provided the conditions were reasonably dry, the apparatus worked well, though, as before, the sensitivity of the Kunz cell varied somewhat from day to day.

In accordance with previous laboratory experience it was assumed that the sensitivity of the *K*-cell was constant throughout a test, unless too large a current was passed through it. The readings were thus assumed to be proportional to the light, so that the light at any depth could be compared with the light just above or just below the surface. Simultaneous readings of the deck photometer gave the variations in the incident light, so that the absorption due to the water could be found. As already mentioned it was found best to limit the voltage applied to the *K*-cell to 20 volts for full daylight. When such a depth had been attained that the current through the cell had fallen to about 100 scale divisions (0·2 microampere) the voltage was raised to 120 volts, thus increasing the current about fourteen times. Alternate readings for voltages 20 and 120 at this and, possibly, neighbouring depths allowed this voltage factor to be found, so that the readings with the higher voltage at greater depths could be compared with the surface readings.

As the chief object of these tests was to gain experience of the working of the apparatus, the measurements at different depths were not made in regular order. Since large variations in daylight often occur with surprising rapidity, and as it was not possible to read both photometers absolutely simultaneously, the readings were made alternately at the briefest possible intervals, a few seconds elapsing between each, and means taken over chosen periods for which the light was found to be approximately constant. When continuous irregular variations occurred, so that approximate constancy was unattainable, a large number of readings were taken in each set so as to smooth out the effect of rapid fluctuations of light. Each photometer reading shown in the table is such a mean, the time entered being the average clock time of the set.

One disadvantage of the telephone method of measurement soon made itself apparent. If the sun is shining and there are any appreciable waves, the light just below the surface is so unsteady that a reliable balance is almost impossible. This makes it difficult to measure the light lost by reflection at the surface under these conditions. The trouble quickly decreases with increase of depth, which renders the diameter of the cone supplying light to the photometer larger compared with the length of the waves.

It is obvious that the factors *A*, *B*, *C*, etc., vary with the altitude of the sun, as plotted for direct sunlight only in fig. 3, and also with the proportion of direct sunlight to diffuse skylight. It is proposed in future to make routine tests on this point by observing the readings of the deck photometer when exposed to and screened from direct sunlight respectively. Only a few of these tests have, so far, been made, and those not under the best conditions. How-

ever, in the majority of cases the altitude of the sun was such that there would not be a very great difference between the values for direct and diffuse light. Accordingly a rough estimate, based on the conditions during the observations, has been made of the proportions of direct to diffuse light. This ratio (λ) is entered as 2, 1, or 0 in Table I, which shows the method of reduction of one series of readings taken on September 9th. D, E, F, and A are the values of the various coefficients as read off for direct sunlight from fig. 3, while D', E', F', and A' are the corresponding mean values allowing for the estimated proportion of diffused light. D'' is the slightly modified values of D' used for the submarine photometer in air to allow for the screening effect. T is the mean time (G.M.T.) of a set of readings with the submarine photometer at a depth d metres, a being the corresponding altitude of the sun, which is used for finding D, E, etc. The letters a and b in the depth column mean that the submarine photometer was just above and just below the water surface, respectively. In the first case the window was dry. The deck photometer was always placed in a position on the deck, or on the pier, as free from all shading as possible.

G is the mean reading of the deck photometer, the anode potential being always very close to 120 volts, K_1 and K_2 the readings of the submarine photometer with anode potentials of 20 and 120 volts respectively. The ratio $\frac{K_2}{K_1}$ obtained at some convenient depth, or the mean of the ratios so found for a few depths within the most suitable range, enables the value appropriate to K_1 to be deduced from the value of K_2 at greater depths, where the actual value of K_1 is too small for accurate measurement. These values are entered as K'_1. For the sake of convenience the actual values of K_1 for shallower depths are repeated, so that K'_1 may be taken throughout as the "computed" value of K_1. In the series of readings shown the light never became weak enough for this method to be advantageous, but it is shown as an example.

I_a is the illumination in air in thousands of metre candles, obtained by multiplying the product $D'G$ by 0·195. The difference between the illumination on the deck or pier and that just above the water surface is disregarded. It would generally be small. I is the illumination recorded by the submarine photometer on the assumption that when the latter is above water $I = I_a$ $p = \frac{100\,I}{I_a}$. $f = \frac{p}{A'}$, i.e. it represents the percentage that the illumination at the given depth is of the theoretical illumination just below a smooth water surface. For the reason already given the illumination just below the actual water surface is frequently hard to measure. $F'd$ is obviously a measure of the optical depth, and is used in calculating the absorption coefficient per metre (μ), as shown on Table 2 which shows the more reliable of the results obtained. These are arranged in series of increasing depth.

The values of μ given in the last column are calculated from the ratios of f for the depths immediately above and below. In cases where two sets of readings were made at approximately the same depth their results are averaged as shown. In some cases, marked with a star, the value of μ so found includes the effect of possible differences between the actual loss of light at the water surface, and that calculated for a smooth surface.

TABLE I.

T	1·40	2·2	2·5	2·21	2·35	2·43	3·0	3·8	P.M., G.M.T.
α	41°	40°	40°	38°	36°	35°	33°	32°	
λ	2	1	2	1	1	1	1	1	
d	a	6·6	6·6	5·2	3·85	2·35	1·1	b	metres.
G	328	187	368	204	330	256	244	253	$\times 10^{-4}$ volt.
K_1	917	54	96	85	212	264	396	759	„ „
K_2	—	726	1352	1159	—	—	—	—	„ „
$\frac{K_2}{K_1}$	—	13·45	14·1	13·65 (mean 13·7)		—	—	—	
K'_1	917	53·0	98·7	83·8	212	264	396	759	$\times 10^{-4}$ volt.
D	1·56	1·59	1·59	1·67	1·76	1·81	1·91	1·97	
E	1·17	1·175	1·175	1·195	1·215	1·225	1·245	1·255	
F	1·21	1·225	1·225	1·24	1·26	1·27	1·29	1·30	
D'	1·75	1·86	1·77	1·90	1·94	1·97	2·01	2·04	
E'	1·16	1·16	1·17	1·17	1·18	1·19	1·20	1·21	
F'	1·20	1·21	1·21	1·21	1·22	1·23	1·24	1·25	
$D'G$ ...	574	348	651	388	640	505	490	516	$\times 10^{-4}$ volt.
$D''K'_1$ ...	1610	—	—	—	—	—	—	—	„ „
$E'K'_1$ ...	—	61·5	116	98	250	314	475	918	„ „
I_a	112	68	127	75·5	125	98·5	95·5	100·5	$\times 10^3$ metre candles.
I	112	4·3	8·0	6·8	17·5	22	33	64	„ „ „
p	100	6·35	6·3	9·0	14	22·5	34·5	63·5	per cent.
$100A$ ...	—	76	76	73·5	71	70	67	65	„ „
$100A'$ ...	—	66	69	65	63·5	63	61·5	60·5	„ „
f	—	9·6	9·1	14	22	35·5	56·5	105	„ „
$F'd$ ...	—	8·0	8·0	6·3	4·7	2·9	1·4	0·0	metres.

Discussion of Results.

As these experiments were purely of a preliminary nature, no great weight can be attached to any of the results. They were, however, regarded as being sufficiently satisfactory to demonstrate that the method may be used successfully at sea in fine weather. The illumination at any point in the water varies so rapidly with the depth, and very often with time also, that very accurate measurements are neither possible nor necessary. As each absorption coefficient given above is obtained from the ratio of two sets of tests only, without any attempt being made to smooth out irregularities, and as a considerable time frequently elapsed between these sets, it is not surprising that they exhibit considerable variations. The daily mean values range from about 0·7 on September 8th, when the water was obviously obscured by sand to about 0·25 on September 10th. Thus on September 8th an increase of depth of 10 cm. corresponded to a fall of illumination of 7 per cent.—a figure which shows how useless it is to attempt accurate determinations at sea in any but the calmest of weather—while a depth of one metre would reduce the light to one half and 6·6 metres to 1 per cent. On September 10th the corresponding figures were 2·5 per cent., 2·8 metres and 18·3 metres respectively. These figures, of course, only refer to the shallow in-shore waters off Cawsand pier. The limits of depth at which satisfactory readings can be obtained with this apparatus in the English Channel have still to be ascertained.

These values of the absorption coefficient are in general agreement with those found photographically by Knudsen[1] in the North Sea, but, as might be expected, indicate a much more rapid absorption of light than was found by Shelford and Gail for the clear waters of Puget Sound.

Modifications Suggested by the Experiments.

One difficulty always to be met with in photometric measurements afloat is that of avoiding errors due to the shadow of the ship. On the occasion when the apparatus was used on board, the vessel was lying with her head to the N.W., and the photometer was lowered from the end of the mizzen boom which was swung out over the port quarter. The ship thus only intercepted some light from the north sky. This arrangement, however, is in general objectionable, firstly, since any rolling of the vessel causes large variations in the depth of the photometer, and secondly, because the vessel would in general cast less shadow in a fore and aft direction. Accordingly it is proposed to lower the photometer from a spar projecting over the stern. By keeping the vessel's head in a suitable direction serious shading will then be avoided.

It was also found that even slight rolling of the vessel caused very noticeable variations in the reading of the deck photometer, owing to the varying angle of obliquity. Accordingly this photometer has been mounted on gimbals for future use.

During the above experiments the absence of "dark current" was tested occasionally. It is, however, liable to make its appearance rather rapidly if the atmosphere becomes moist, so that in future it is intended to test for it much more frequently. For simplicity, in these initial experiments, no strings were attached to the marine photometer shutter, so that it had to be hauled up to test the dark current. This will be altered in future.

The window of the deck photometer has been altered, as already mentioned,

[1] Conseil Permanent International pour l'exploration de la mer. Publications de Circonstance, No. 76.

Table II.

Date. Remarks. Time of High water.	T P.M.	α	Light	λ	d metres	F'd metres	I_a m.c. $\times 10^3$	I m.c. $\times 10^3$	p %	100A' %	f %	μ $(m.)^{-1}$
	2·41	36°	bright sun	2	*a*	0	128	128	100	—	—	
5/9/24 Calm. 9.27 A.M.	2·49	35°	„ „	2	*b*	0	120	71·5	59·5	65·5	91	0·39
	2·56	34°	„ „	2	1·1	1·4	114	39	34	64·5	52·5	0·375
	3·4	33°	„ „	2	3·45	4·35	113	12·5	11	63	17·5	
	12·7	45°	sun through	1	*a*	0	112	112	100	—	—	0·705*
	12·12	45°	light broken	1	1·1	1·3	108·5	30	27·5	68·5	40	0·46
	12·24	45°	clouds	1	2·15	2·6	129	19·5	15	68·5	22	
	1·43	42°	light clouds	1	4·8	5·8	78	0·38	0·49	67	0·73	0·51
8/9/24 Slight sea; much sand in water. 12.34 P.M.	1·3	44°	„ „	1	5·8	6·9	122·5	0·425	0·345	68	0·415	
	1·10	44°	„ „	1	5·8	6·9	101	0·21	0·21	68		
	2·7	40°	cloudy	0	2·15	2·55	63	6·6	10·5	56	19	0·81
	1·53	41°	„	0	3·0	3·6	67	3·0	4·5	56	8·0	
	4·53	20°	cloudy	0	*a*	0	27·5	27·5	100	—	—	1·00*
	4·58	19°	„	0	1·1	1·3	14	1·55	15	56	27	
	12·48	44°	light clouds	1	*a*	0	70	70	100	—	—	0·345*
	12·57	44°	„ „	1	1·1	1·3	120	52·5	43·5	68	64	0·35
9/9/24 On s.s. Salpa. Fresh N.W. wind. 1.27 P.M.	2·30	37°	„ „	2	6·2	7·6	148	7·0	4·75	67	7·1	0·445
	1·18	44°	„ „	1	8·35	9·9	55·5	0·87	1·6	68	2·55	
	1·27	43°	„ „	2	8·35	9·9	159·5	3·1	1·95	71		0·265
	1·52	41°	„ „	1	10·25	12·3	56·5	0·54	0·96	66·5	1·37	
	1·58	40°	„ „	1	10·25	12·4	76	0·65	0·86	66		
	1·40	41°	light clouds	2	*a*	0	112	112	100	—	—	
	3·8	32°	„ „	1	*b*	0	110·5	64	63·5	60·5	105	0·445
	3·0	33°	„ „	1	1·1	1·4	95·5	33	34·5	61·5	56·5	0·31
	2·43	35°	„ „	1	2·35	2·9	98·5	22	22·5	63	35·5	0·265
	2·35	36°	„ „	1	3·85	4·7	125	17·5	14	63·5	22	0·27
	2·21	38°	„ „	1	5·2	6·3	75·5	6·8	9·0	65	14	0·24
10/9/24 Smooth. 2.51 P.M.	2·2	40°	„ „	1	6·6	8·0	68	4·3	6·35	66	9·45	
	2·5	40°	„ „	2	6·6	8·0	127	8·0	6·3	69		
	5·0	16°	cloudy	0	*b*	0	32	15·5	48·5	56	86·5	0·19
	4·51	18°	„	0	1·1	1·3	37·5	14	38	56	68	0·275
	5·14	14°	„	0	2·2	2·6	23	6·1	26·5	56	47·5	0·31
	5·27	12°	„	0	3·65	4·35	9·85	1·55	15·5	56	27·5	0·165
	4·37	19°	„	0	6·8	8·1	37·5	3·1	8·3	56	14·8	

so that it resembles that of the submarine photometer more closely. This has, as was expected, somewhat reduced its sensitivity.

Finally, it was considered that more confidence would be felt in the readings of the Kunz cell if some method were at hand for quickly standardising it under various illuminations.

Screens for Standardising the Kunz Cell.

A set of three "dark" glass screens has been obtained of various degrees of opacity and as nearly as possible of a neutral tint. The transmission factors of these screens and of their combinations in pairs have been measured for artificial light [a Mazda "Daylight" gas-filled bulb] by means of the deck photometer (*G*-cell), the currents being compared by means of a sensitive galvanometer, or a quadrant electrometer shunted by a set of mica condensers ranging from 0·05 to 1·0 microfarad, according to their magnitudes. The reduction of light in passing through a screen is due partly to genuine absorption and partly to reflection losses. If the screen were absolutely non-selective, and its refractive index were known, we could, if we knew the transmission factor for any angle of incidence, calculate the factor for any other angle. These conditions are, however, probably not easy to realise in practice, as most screens exhibit some selectivity, and the refractive index of the surface layers, which governs the reflection losses, may differ from the value for the screen as a whole. The screens actually used were examined with a small spectroscope. The darkest one, which appears slightly greenish, shows a slight absorption band in the red and a region of exceptional transparency in the yellowish green. These effects are most noticeable when all three screens are superposed, so they are probably also present, though hardly detectable, in the lighter screens also. No traces of anomalous absorption could be detected in the blue-green, blue, and violet parts of the spectrum, which are the important regions as far as photo-electric work is concerned.

It is evidently necessary to test the transmission factors of the three screens and their combinations at various angles. This was carefully done, precautions being taken to eliminate, or correct for, all obvious sources of error. It was found that the screens were certainly not non-selective, the transmission factor for a given angle for the combination of two screens exceeding the product of the separate factors. Small differences were also found between the calculated and the observed effect of obliquity. Curves were plotted showing the results for the various screens and combinations at various angles, and from them the transmission factors for diffused light were calculated. These were compared with the factor for diffuse daylight, as found with the photometer exposed on a window-sill so that it received light from approximately all the western half of the sky, which did not contain the sun. The transmission factors thus obtained for the various screens and combinations averaged about 10 per cent. higher than those found by calculation from the direct illumination experiments with the "Daylight" lamp. This difference is probably chiefly ascribable to want of uniformity of the sky, which was distinctly brighter overhead, i.e. in the region of small angles of incidence. The approximate correspondence shows that there is no large difference in the transmission factors for daylight and the "Daylight" lamp.

It is proposed to use these screens for frequent tests of the sensitivity of the submarine photometer at various illuminations and at different anode potentials.

SUMMARY AND CONCLUSION.

A method is described of using photo-electric cells for submarine photometry which may be employed in a comparatively small vessel at sea in fine weather. The photo-electric current is passed through a known high resistance, the P.D. between the ends of the latter being balanced against a potentiometer. A telephone is used as a detector instead of a galvanometer, thus rendering a steady support unnecessary. In order to enable this to be done the telephone circuit is interrupted many times per second by a special form of interrupter. By the interposition of a two-stage valve amplifier between this circuit and the telephone, the sensitivity is rendered ample for the purpose required. A vacuum photo-electric cell is used as a standard, and also for recording fluctuations in the surface light, while the light below the surface is measured by a cell of the Kunz type.

In order to deduce the actual illumination from the photometer reading it is necessary to multiply by a factor correcting for the effect of obliquity and the loss of light at the front surface of the photometer window. This factor varies with the direction of the illumination and with the proportion of direct to diffused light. Different factors must be used according as the photometer is in air or in water.

Preliminary tests carried out with the apparatus at Cawsand Bay, near Plymouth, have yielded results of a distinctly promising nature. The mean absorption coefficients per metre for shallow water varied from 0·7 on a day when the water was obviously sandy to 0·25 in calm weather a few days later.

It is hoped in the near future to make numerous measurements by this means in conjunction with Dr. W. R. G. Atkins.

The work has been carried out under the auspices of the Marine Biological Association of the United Kingdom, who have most kindly provided the funds to defray the cost of a portion of the apparatus and the incidental expenditure, in addition to providing laboratory and marine facilities at Plymouth. I wish to record my gratitude to the Director, Dr. E. J. Allen, for his most helpful interest and assistance throughout.

The laboratory work has chiefly been done in the Physical Laboratory, Trinity College, Dublin, and my thanks are also due to Professor W. E. Thrift for laboratory facilities and for the loan of the Kunz cell and other apparatus.

Finally, I must thank Professor Joly and Professor H. H. Dixon for the loan of some of the apparatus used.

No. 10.

THE VARIATIONS IN THE QUANTITY OF FOOD REQUIRED BY CATTLE FOR MAINTENANCE AND PRODUCTION WITH DIFFERENT KINDS OF RATIONS.

[Second Paper.]

By J. WILSON, M.A., B.Sc.

[Read June 23. Printed July 31, 1925.]

In the first paper on this subject it was shown that the efficiency of a ration varies with the kind and quantity of long fodder it contains, and that Kellner's estimates for maintenance and production need revision. The purpose of this paper is to show that Armsby's estimates, which were arrived at by a different method, also need revision.

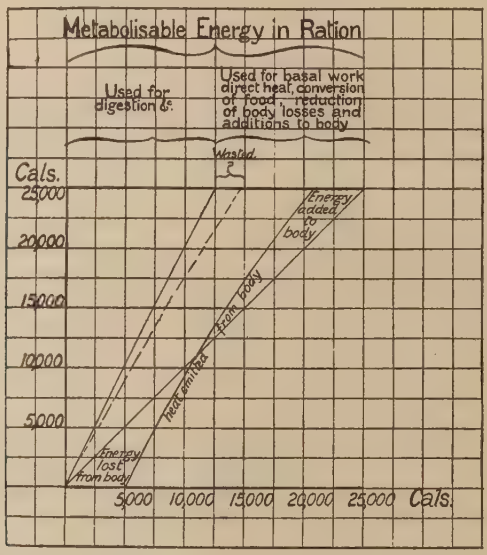
A starving animal lives by converting the potential energy in its body indirectly and directly into heat. Some of its energy stores are decomposed, the energy released activates the muscles of the heart, lungs, and other internal and excretory organs, and, having done so, gradually escapes from the body as heat. But, since the animal's muscles are incapable of activity, unless within certain temperatures, the energy so released may be insufficient to maintain the body within those temperatures and more potential energy may be decomposed directly.

When food is consumed additional organs come into use, and those in use during starvation have increased work to do. The food must be prehended, masticated, carried to the stomach, raised to the body temperature, and tossed about, disintegrated, and digested. It must then be forced through the intestines, the digested portion absorbed and the undigested expelled. Till the digested portion is absorbed, the call upon the stored up energy is greater than during starvation, and, if some of it be not at once a source of energy but must first be converted into other forms, still more energy is necessary for its conversion, transportation, and deposition; and, for a time, the call upon the previously stored up energy is all the greater. Thus, as the consumption of food increases, more energy is transformed and more directly produced heat set free.

The energy in the digested portion of the food is nearly, but not all, transformable—metabolisable—by the animal. Some of that in the urine is not converted into another form. Thus, the non-metabolisable portion of the energy in the food is that contained in the undigested dung, in the uncombusted marsh gas, and in the digested but incompletely combusted constituents of the urine.

The metabolisable energy in the food may be utilised in several ways. Of necessity, some must always be expended, or replace that just expended, in manipulating, heating, and digesting the food, absorbing and converting that digested, and expelling the dung and other increased excreta, that is, in doing the extra work to be done when food is consumed over and above that to be done during starvation. The remainder may replace or reduce the

energy withdrawn from the body, when the animal is partially starved, or be stored up in the body, when the animal is fed with more food than is necessary to maintain its energy store at a constant level. But some may help to maintain the body temperature, if the heat produced from food manipulation and body metabolism during partial starvation be not enough, while, if more heat be produced at any time than can at once be used, as may happen shortly after a readily digestible meal, the balance will escape rapidly from the body, possibly with no physiological gain to the animal and no economic gain to its owner.[1] This may be made clearer by the following diagram in which the direction of the dotted line is, of course, very speculative. Probably this line ought to be sometimes to the left, sometimes to the right, of the continuous line beside it.



From this diagram, it is obvious that, if the energy required for food manipulation, digestion, and excretion, for internal work, and heat, and that which may be wasted be regarded as the energy required for maintenance, then maintenance energy = metabolisable energy – energy retained (+ or –), and, since metabolisable energy = gross food energy – non-metabolisable energy, maintenance energy = gross food energy – non-metabolisable energy – energy retained. Kellner used the last of these equations. He fed his animals with rations known to be very close to those necessary for idle maintenance, measured the gross and the non-metabolisable energy in the rations, subtracted the latter from the former, and, having made allowance for the energy retained (+ or –) by the animal, determined what was necessary for maintenance. To determine the relative nutritive values of different foods and food nutrients, he added measured quantities of them to presumptive maintenance rations and found how much of each food or food nutrient was required to produce a pound of fat.

[1] This is the phenomenon which human and small animal physiologists speak of as "specific dynamic action."

As shown in the previous paper, Kellner's estimates for maintenance and fat production are inaccurate, because he had not observed that the food required for either kind of work varies seriously with the kind and quantity of long fodder in the ration.

Armsby found what is required for maintenance by measuring with a calorimeter the heat emitted by the animal and the relative nutritive values of foods by determining what he called their net metabolisable energy. It is not clear that he allowed for the energy required to heat the food and drink to the temperature of the body. If he did not do so, his estimates for maintenance are slightly too high and for nutritive values slightly too low; but, since his method fails, as Kellner's failed, and for the same reason, the exact discrepancy is immaterial.

Since he could not feed his bullocks so that they should neither gain nor lose energy, any more than Kellner could, Armsby fed them during two different periods with different quantities of the same ration, both insufficient for idle maintenance, and, from the figures obtained, calculated what was required for both idle and starving maintenance. For example, the following figures were obtained from a bullock fed during two different periods with different quantities of meadow hay:—

	Dry matter of hay eaten.	Metabolisable energy.	Heat produced.	Energy withdrawn from the body.
	lbs.	Cals.	Cals.	Cals.
Period 3	6·17	5768	8064	2296
Period 4	10·21	9544	9812	268
Difference	4·04	3776	1748	2028
per pound of dry matter eaten		935	433	502
per metabolisable Calorie ...			·463	·537

From this it was calculated that, since 4·04 lbs. of dry matter added to ration 3 reduced the energy withdrawn by 2028 Calories and, therefore, 1 lb. reduced it by 502 Calories, the addition to ration 4 of ·53 lb., i.e. 268/502, of dry matter, would reduce the energy withdrawn by 268 Calories, and make the exact maintenance ration 10·74 lbs. If the calculation were in Calories of metabolisable energy, then, since 1 Calorie of metabolisable energy prevents the withdrawal of ·537 Cal. from the body, as many metabolisable Calories as would prevent the withdrawal of 268 Calories from the body, namely, 499, i.e. 268/·537, would have to be added to 9544; and the bullock's maintenance ration should contain 10,043 metabolisable Calories.

The energy withdrawn from the bullock's body when starved is calculated as follows:—Since the reduction of ration 4 by 4·04 lbs. of dry matter reduced the heat produced by 1748 Calories and its reduction by 1 lb. would, therefore, reduce the heat produced by 433 Calories, the withdrawal of the whole ration of period 3 would reduce the heat production by 2672 Calories, and leave 5392 to be furnished from the body. Calculating by Calories: since the reduction of the ration of period 4 by 1 metabolisable Calorie reduces the heat produced by ·463 Calorie, the withdrawal of ration 3 would reduce the heat produced by 2673 Calories, i.e. 5768 × ·463, and leave 5391 to be furnished by the body.

But this method assumes that the proportion of metabolisable energy extracted from the ration and used for food manipulation, internal work, and heat is the

same for all sizes of that ration—that the bullock from which the above results were obtained required no more proportionate energy for maintenance when fed with 10·21 than when fed with 6·17 lbs. of dry matter, and that he would require no more in proportion when fed with 10·74 and no less when fed with 1 or even with ·1 lb. It assumes that, if the bullock were fed with any number of different quantities of the same ration, the same result would be got from any two: that, if the results got from all the readings were plotted, the plottings would all fall upon the same straight line.

For their paper on the "Net Energy Values of Feeding Stuffs for Cattle,"[1] Armsby and Fries fed a number of bullocks during more than two periods with different quantities of the same foods or food mixtures, and the maintenance rations for these bullocks, calculated from these feedings, taken two at a time, are not the same. For example: in experiment 208, bullock *C* was fed with different quantities of alfalfa hay during three different periods, and the following data obtained:—

Period.	Live weight.	Energy in Ration.			Heat emitted.	Energy withdrawn from the body.
		Total.	Non-Metabolisable.	Metabolisable.		
	cwts.	Cals.	Cals.	Cals.	Cals.	Cals.
4	5·68	19700	11313	8387	9052	665
5	5·58	13657	7992	5665	7357	1692
6	5·4	9336	5228	4048	6124	2076

From these three sets of figures taken two at a time the metabolisable energy required by the bullock for maintenance may be calculated as follows:—

Periods used.	Increased metabolisable energy in larger ration.	Reduced energy withdrawn from body with larger ration.	Reduced energy withdrawn per metabolisable Calorie.	Energy withdrawn with larger ration.	Metabolisable energy necessary to prevent this withdrawal.	Metabolisable energy necessary for maintenance.
	Cals.	Cals.	Cals.	Cals.	Cals.	Cals.
4 & 5	2722	1027	·377	665	1764	10151
4 & 6	4339	1411	·325	665	2046	10433
5 & 6	1617	384	·237	1692	7140	12805

The energy necessary for starving maintenance may be calculated as follows:—

Periods used.	Reduced metabolisable energy in smaller ration.	Reduced heat production.	Reduced heat production per metabolisable Calorie.	Reduced heat production if smaller ration were withdrawn.	Energy necessary for starving maintenance.
	Cals.	Cals.	Cals.	Cals.	Cals.
4 & 5	2722	1695	·623	3529	3828
4 & 6	4339	2928	·675	2732	3392
5 & 6	1617	1233	·762	3084	3040

[1] Journal of Agricultural Research, vol. iii, pp. 435–491.

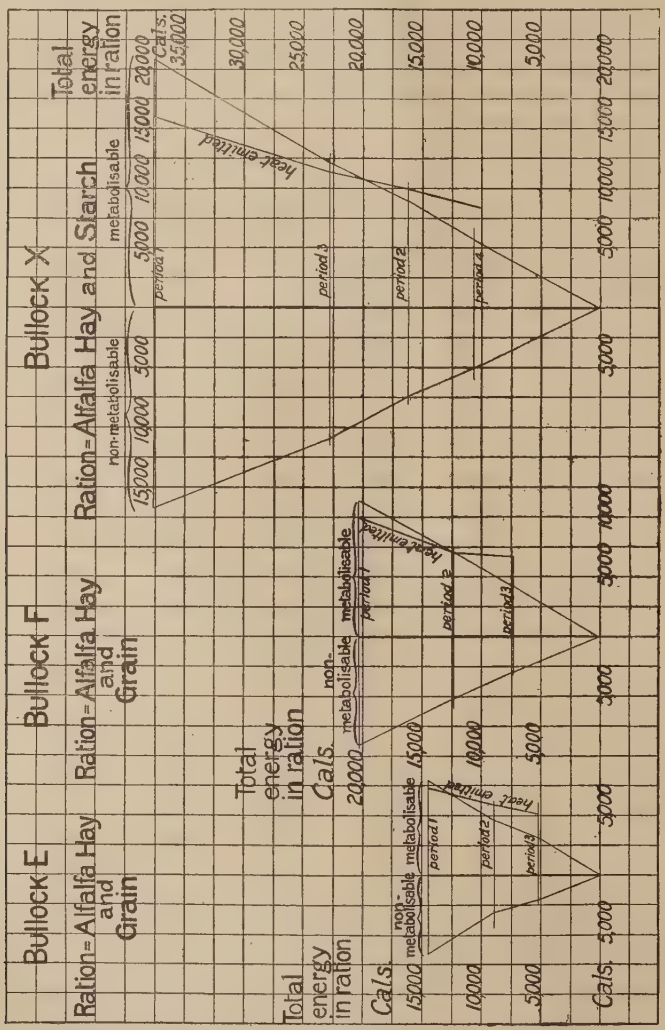
Obviously, a method which gives such variable results cannot be sound. Since the ration fed in period 4 is nearest what is necessary for idle maintenance and that in period 5 next nearest, the result got by calculating from these two periods may be assumed to be the best. On this assumption, the results got by combining periods 4 with 6 and 5 with 6 are out by about 2·8 and 28 per cent. respectively. And if periods 5 and 6 give the best result for starving maintenance, the results got by combining periods 4 with 6 and 4 with 5 are out by about 11 and 26 per cent. respectively.

The following data were obtained from three other bullocks, each of which was fed for three periods on different quantities of alfalfa hay, and for three or more periods on different quantities of equally proportionate mixtures of alfalfa hay and concentrates:—

Bullock.	Food.	Period.	Live weight.	Energy in Ration.			Heat emitted.	Energy withdrawn from body.
				Total.	Non-metabolisable.	Metabolisable.		
			cwts.	Cals.	Cals.	Cals.	Cals.	Cals.
E	Alfalfa hay ...	4	4·4	18390	10488	7902	7879	– 23
		5	4·22	10612	6333	4279	5764	1485
		6	3·86	6226	3626	2600	4566	1966
F	Alfalfa hay ...	4	6·3	26912	15509	11403	11474	71
		5	6·05	15416	8794	6622	8007	1385
		6	5·75	9634	5799	3835	6781	2946
X	Alfalfa hay ...	5	7·93	35200	10101	15099	13248	–1851
		6	7·92	26564	14609	11955	11226	– 729
		7	7·4	15179	8377	6902	8864	1962
E	Alfalfa hay and	1	4·12	14535	6541	7994	7483	– 511
	grain	2	4·06	7950	3227	4723	5924	1201
		3	3·52	5216	2122	3094	5084	1990
F	Alfalfa hay and	1	5·91	20381	9022	11359	9888	–1471
	grain	2	5·75	12446	5115	7331	7115	– 16
		3	5·55	7441	3003	4438	6734	2296
X	Alfalfa hay and	1	7·63	37639	16778	20861	16015	–4846
	starch	3	7·6	22817	10799	12018	11361	– 657
		2	7·2	16148	7290	8858	9920	1062
		4	7·	10580	4953	5627	8404	2777

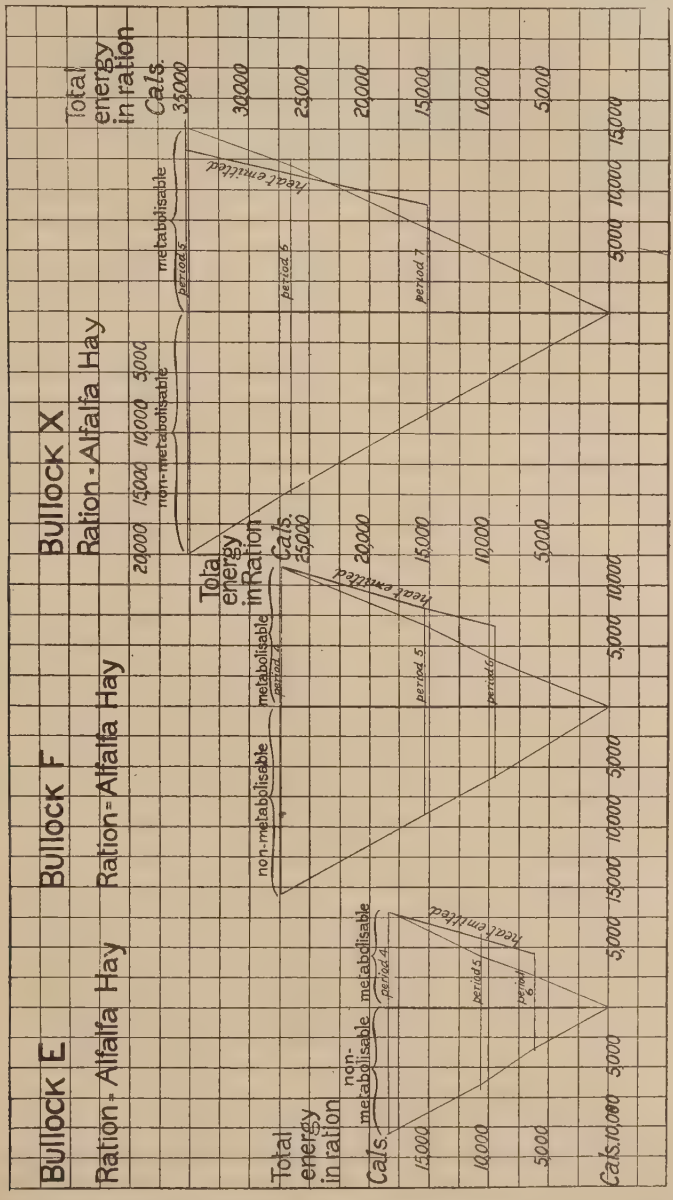
If these data be plotted, as in the following diagrams, the reason for the unsoundness of Armsby's method becomes apparent. Neither the plottings for metabolisable energy, with different sizes of rations, on the one hand, nor for heat emitted, on the other, fall on straight lines. Even the plottings for non-metabolisable energy do not fall on straight lines: though small rations are generally better digested than large ones, the very small ration is not better digested than one slightly larger. The plotted points form three curves which could be drawn with sufficient accuracy, were enough observations made, and the curve for heat emission, that is for maintenance, falls more and more

to the right, the farther it leaves its base, especially so after idle maintenance is passed and the animal is storing up energy. Thus, with two observations only, Armsby's method can never give complete accuracy. For idle maintenance, its best approximate result would be given with two rations close up to what is actually required; for starving maintenance, with two rations as near as possible to zero.



The diagrams also show that, if the points where the heat emission curves cross the metabolisable energy curves be taken as indicating approximately what is required for idle maintenance, the three bullocks, *E*, *F*, and *X*, required

more energy for maintenance when fed with hay alone than when fed with hay and concentrates. The differences in the bullocks' weights when fed with



hay, on the one hand, and with hay and concentrates, on the other, are not enough to make a serious difference in their maintenance requirements.

Armsby found the relative nutritive values of foods by determining what he called their net energy value, which he defines as "the net effect of the food either in causing a storage of chemical energy in the form of fat, protein, etc., in the body, or, in the case of a submaintenance ration, in diminishing the amount of energy which must be supplied by the katabolism of body tissue."[1]

By the "net effect of the food" Armsby means the net effect of the metabolisable portion of the food. An example of his method of calculating it is to be found on page 119 of this paper, where it is shown that 3776 Calories of metabolisable energy have the effect of preventing the withdrawal of 2028 Calories from the bullock's body. The net energy of the ration is therefore 53·7 per cent., i.e. 2028 × 100/3776. The net energy of the ration per metabolisable Calorie is, therefore, ·537.

If we calculate, in the same way, the net energy of the rations fed to bullocks *E*, *F*, and *X*, we shall have some indication of how far the method is reliable, thus:—

Bullock.	Food.	Periods used.	Extra metabolisable energy in larger ration.	Energy stored or saved by larger ration.	Net energy per metabolisable Calorie.
			Cals.	Cals.	Cals.
E	Alfalfa hay ...	4 & 5	3623	1508	·42
		4 & 6	5202	1889	·36
		5 & 6	1679	481	·29
F	Alfalfa hay ...	4 & 5	4781	1314	·28
		4 & 6	7658	2875	·37
		5 & 6	2787	1561	·56
X	Alfalfa hay ...	5 & 6	3144	1122	·35
		5 & 7	8197	3813	·46
		6 & 7	5053	2691	·53
E	Alfalfa hay and grain	1 & 2	3271	1712	·52
		1 & 3	4900	2501	·51
		2 & 3	1629	789	·48
F	Alfalfa hay and grain	1 & 2	4028	1455	·36
		1 & 3	6921	3767	·54
		2 & 3	2893	2312	·8
X	Alfalfa hay and starch	1 & 3	8843	4189	·47
		1 & 2	12003	5908	·49
		1 & 4	15234	7623	·5
		3 & 2	3160	1719	·54
		3 & 4	6391	3434	·54
		2 & 4	3231	1715	·53

The results are again uneven. The rations containing hay and concentrates give higher net energy values than those containing hay alone. But, if the

[1] Journal of Agricultural Research, vol. iii, p. 436.

results found with bullock *E*, which did abnormally well on very small rations, be excepted, there are clear differences between the energy values found with larger and smaller rations, and, with hay rations especially, the differences are so great that fair averages could not be struck from the observations. Thus, if idle maintenance is to be determined accurately, whether by Kellner's method or by Armsby's, it must be determined for every food and every food mixture separately, with rations approximating closely in size to those actually necessary; and the nutritive value of a food as expressed in Kellner's starch equivalent or Armsby's net energy-values gives the nutritive value of that food only when fed in a combination similar to that by which its value was determined.

No. 11.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1924.

[Read May 26. Printed July 28, 1925.]

The quantity of emanation issued was 12,885 mc., as compared with 12,797 mc. in 1923, and 9,283 m.c. in 1922. The stock of radium was increased early in the year by the acquisition of a further 51 m.g. of the element. This, as mentioned in the last report, was purchased by the Society out of the proceeds of the sale of an old laboratory stock of platinum. It has proved of the greatest value in helping to supply the increasing demand for large doses of emanation.

The following are reports received from some of the largest users of emanation.

1.

Cases of Cancer of the Uterus treated with Radium Emanation.—The dosage is indicated as the primary number of millicuries introduced and the number of hours' application. In those cases where the emanation had passed its maximum, the table figure of value $\frac{8}{1000}$ is shown in front of the original number of millicuries and the hours. The final figure given as dose is the millicurie-hours, according to the value of the emanation in the middle of the application.

Case 21984, aged 58.—Cancer of Body of Uterus, extending to Cervix. Marked infiltration of Left Vaginal Fornix and Vagina. All the parts very fixed. Considered inoperable. Profuse foul discharge.

Radium Application, September 18th, 1923—142 millicuries for 24 hours. Dose, 3144 millicurie-hours. Screen, Institute silver holder with half-inch gauze spacing.

2nd Radium Application, March 12th, 1924— $\frac{7 \cdot 68}{1000} \times 127 \cdot 5$ millicuries for 24 hours. Dose, 2143 millicurie-hours.

Patient feels and looks much better. Very little discharge, and scarcely any pain.

Report, July, 1924.—General condition bad; local condition not causing much inconvenience, but evidently in a terminal state.

Case 22000, aged 66.—Marked senile atrophy of pelvic organs, but Body of Uterus enlarged. Symptoms, constant bleeding. Curettage for diagnosis. Pathological Report—Solid Cancer.

September 11th, 1923—Vaginal Hysterectomy.

Radium Application, September 21st, 1923—Operation site perfectly healed; 142 millicuries for 12 hours. Dose, 1627 millicurie-hours.

Report, March 18th, 1924—In perfect health. No local symptoms.

Case 22008, aged 30.—Mass growing from Cervix very friable. Uterus and parametrium mobile. Slight thickening running from Cervix to right Sacro-uterine fold. Pathological Report of section—Carcinoma.

September 12th, 1923—Wertheim's Hysterectomy, as wide as possible, and taking practically the whole Vagina.

Radium Application, September 22nd, 1923—Operation healed perfectly; 130·7 millicuries for 12 hours. Dose, 1488 millicurie-hours.
Report, March 22nd, 1924—In perfect health. No local symptoms.

Case 22023, aged 34.—Friable mass from Cervix. Uterus mobile. Mass removed. Pathological Report—Carcinoma.
October 9th, 1922—Wertheim's Hysterectomy, extending well down Vagina.
Radium Application, October 29th, 1922—Operation well healed, except for bladder fistula; 57·8 millicuries for 24 hours. Dose, 1272 millicurie-hours.
2nd Radium Application, September 19th, 1923—Mass of induration surrounding remains of Vagina, and extending out to Pelvis. Parts very fixed. General health greatly improved. Fistula persisting. $\frac{8\,3\,5}{1\,0\,0\,0}$ × 142 millicuries for 24 hours. Dose, 2592 millicurie-hours.
Later Report—The patient had been in Hospice for the Dying up to 2nd application, but left subsequently, as she was so well; there was still marked induration of parts, although discharge was very slight, and possibly only due to fistula. Lost sight of.

Case 22172, aged 35.—Cervix friable, bleeding. Body of Uterus normal. Parts quite mobile, and no evidence of extension.
February 18th, 1924—Hysterectomy. Total, but not taking the parametrium, and only a small portion of the Vagina.
Radium Application, March 13th, 1924—Operation almost healed, except for area to one side of vaginal fornix. $\frac{8\,9\,7}{1\,0\,0\,0}$ × 127·5 millicuries for 16 hours. Dose, 1343 millicurie-hours.
There was little improvement; the unhealed portion of Vagina seemed to be cancerous growth, and progressively extended.
July, 1924—Condition bad, with considerable extension.

Case 22203, aged 39.—Cervix a friable fungating mass, with marked extension and fixity in the parametrium, so that Fundus of Uterus could not be defined.
Radium Application, March 11th, 1924—127·5 millicuries for 24 hours. Dose, 2808 millicurie-hours.
July, 1924—The fungating mass had considerably cleaned, but the induration persisted extensive. Abdominal Hysterectomy. Only the Body of Uterus capable of removal, owing to old pelvic adhesions and extensive induration of parametrium.
October, 1924—General condition much improved, and bleeding and discharge ceased.

2.

As the radiations emitted by radium and its emanation have similar actions on the tissues, for the sake of brevity, the term "radium" has been employed throughout.

Rodent Cancer.—The moist variety is less common than the dry type. Of twenty-seven cases treated during the past year, twenty-two were of the dry indurated type.

Five of the latter had multiple ulcers, and four had ulcers on the eyelids.

Cases treated,	...	...	27
Ulcers healed,	...	...	23
Still under treatment,	...	...	4

Recurrence of the ulcer following radium treatment is rare, if the dosage and filtration have been properly estimated.

Oral Carcinoma.—Four cases of this group had carcinoma of the lower lip. Two were early, superficial, and small, presenting the characteristic appearances and history, that is a small superficial ulcer covered by a scab, which falls off at intervals. There was no enlargement of the submaxillary or cervical lymphatic glands. To both of these patients were given surface applications of sixty to seventy millicurie-hours. The ulcers have completely healed.

Two were recurrent ulcers: one in a man aged 75, from whose lip an ulcer was excised thirty-five years ago. The ulcer, which was 2·5 cm. × 1·5 cm., was in the middle line, close to the scar of the original operation. It appeared from three to four years ago. It was superficial, but deeper in the centre than at the margins, which were nodular. There were no enlarged glands.

A surface application was given, and three weeks later needles were embedded in the lip. This was followed by the application of a "full strength" plaque, 5 × 7·5 cm., with a filter of 1 mm. lead, 2 cm. distance. This plaque was applied to three separate areas over the submaxillary and cervical lymphatic glands. The ulcer healed completely. The patient, who was addicted to alcohol, died about three months later from an intercurrent disease.

The second recurrent case was a man aged 58, from whose lower lip an ulcer was excised nine years ago. A surface application was given to this ulcer. It is now replaced by a soft pliable scar, which is quite healthy.

The remaining cases of this group were:—(1) A female, aged 74, who had a nodule the size of a hazel-nut on the inner surface of the cheek, which recurred three months after operation. On microscopic examination the original tumour was stated to be "fleshy and cellular, with marked leucocytic infiltration and some hæmorrhage." The pathologist stated he had no doubt but the growth was malignant. Needles were embedded in the growth, followed by a surface application externally, both locally and to the cervical lymphatics. The tumour disappeared, but it recurred within seven months, and it again responded to radium treatment. This patient died ten months after the first application of radium from congestion of the lungs.

(2) A female, aged 68, who had a large fungating epithelioma on the upper lip, much of which had been destroyed by the application of an arsenical paste. There was a temporary improvement following treatment by radium, but later reports were not satisfactory. This was a hopeless case from the outset, and she is probably dead.

(3) A female, aged 37. Myxoma of the maxilla. Following excision of portion of the maxilla, the cavity was packed with a tube of radium emanation filtered through ·5 mm. of brass for 550 millicurie-hours. So far there has been no recurrence, and the patient is very well.

(4) Male, aged 60. Epithelioma of the inner surface of the cheek. Result of treatment not known.

(5) Male, aged 37. Carcinoma of the floor of the mouth and base of tongue. When seen one month after onset the case was inoperable. The ulcer was growing rapidly, and it bled very easily. It was almost completely healed two months after treatment by radium, but the extensive enlargement of the cervical glands, which was present when the patient came under observation, was not benefited by surface applications; they continued to grow rapidly.

This patient is now having "deep therapy" after the Erlangen technique, but it does not seem possible to check the progress of the disease.

Uterine Carcinoma.—1. Multipara, aged 41. Four months after amputation of the cervix she had a local recurrence, which was treated in January, 1924, by

implanting needles; the total dose given was 1140 millicurie-hours. Two months after treatment there was no evidence of any lesion, and she is now apparently quite healthy.

2. Multipara, aged 59. A large sloughing crateriform excavation of the cervix, with extension to the left parametrium. Copious offensive discharge. Needles were implanted around the cervix—1277 millicurie-hours. The patient died two months after treatment from metastases.

3. Multipara, aged 45. A large crater in the cervical canal. Os not eroded. Irregular hæmorrhage for nine months. Microscopic sections showed "almost a solid mass of epithelium, with very little connective tissue; very little attempt at acinous formation; fairly numerous mitoses." A 1 mm. brass tube containing radium emanation was passed into the cervical canal—1800 millicurie-hours—in March, 1924. Ten days after there was a smart hæmorrhage. In June the symptoms had disappeared, and the patient was doing well.

4. Multipara, aged 63. This patient was first treated by me in July, 1921, when the uterus was about the size of a coco-nut, and she gave a history of hæmorrhage for two years previously. Needles were embedded around the cervix, and a brass tube (1 mm.) containing emanation was passed into the cavity of the uterus—689 millicurie-hours. In January, 1922, the uterus was about the size of a pear; the cervix was atrophied; the hæmorrhage had ceased, and the patient had put on weight. She had been doing very well until about a month previously, when there was a recurrence of pain and a slight discharge, which was sometimes tinged with blood. An intrauterine application—986 millicurie-hours—was again given. When seen in September, 1922, there was no further reduction in the size of the uterus, and no involvement of the parametrium. Her symptoms then were slight lower abdominal pain, and some frequency of micturition. An intrauterine application of 768 millicurie-hours was then given, and the patient did not report again until April, 1924, when the uterus was about the size of a large orange, and nodular, and adherent to the bladder. There was now a large mass in the left parametrium, and the patient was cachectic. A combined application of embedded needles and a brass tube in the uterus was again given—712 millicurie-hours. This was followed three weeks later by a course of low-voltage X-Ray treatment (9 to 10 Bauer Scale), with a filter of 3 mm. aluminium. A circular field, 10 cm. in diameter, was used at a focal skin distance of 23 cm. Three fields were irradiated suprapubically. A fourth field was irradiated through the perinæum by a method described by me in the Archives of Radiology and Electrotherapy, vol. xxvi, p. 249, January, 1923. An exposure of fifteen minutes was given to each area every fourth day, using 2 milliamperes with a broad focus Coolidge tube. When seen in July, 1924, the uterus was very much smaller. There was atresia of the cervix and vaginal vault. There was no pain, and practically no discharge or hæmorrhage. The patient had put on weight, and cachexia had disappeared. Nothing could now be felt in the left parametrium. An intrauterine application through 1 mm. brass was again given. The patient is doing very well.

5. Multipara, aged 38. Squamous carcinoma of cervix. The os and cervix were eroded, leaving a crater-like ulcer. The patient gave a history of daily hæmorrhage from May to December, 1923, when it ceased for about three weeks, after which there was pain, hæmorrhage, and fœtid discharge. She was wasted and anæmic. In May, 1924, she was given 1800 millicurie-hours through ·75 mm. brass. When seen six weeks later the discharge and hæmorrhage had ceased, and she began to put on weight. The ulcer had quite healed, leaving only a thickened nodule on the left side of the cervix, to which an application

of 486 millicurie-hours through 1 mm. brass was again given. This patient died from metastases toward the end of the year 1924.

6. Multipara, aged 45. Carcinoma of the vulva. The vulva was excised in August, 1923. There was a recurrence in the right inguinal glands five months later. The glands were considerably enlarged and adherent in a mass to the femoral vessels, and there was œdema of the leg. After partial removal of the mass on May 15th a 1 mm. brass tube of emanation was passed deeply into the sinus a week later, and left in situ for 24 hours. This was followed by surface applications filtered through 1 mm. of lead. The total irradiation of the affected area was 1680 millicurie-hours.

7. Multipara, aged 37. Cervix eroded with profuse fœtid discharge. The ulcerated surface bled copiously on touching. Symptoms, four months—anæmia and emaciation. Microscopically "a squamous epithelioma of the cervix, which was rapidly proliferating." On June the 26th she received 1674 millicurie-hours through 1 mm. brass. There was a severe hæmorrhage two weeks later, which was controlled by plugging. Five weeks after treatment the necrotic surface was almost completely healed, and there was no bleeding, and very little discharge; 868 millicurie-hours were again applied. In August the local condition was practically healed, but there was enlargement of the inguinal glands and metastases in the abdomen, and further treatment was abandoned.

8. Multipara, aged 65. Squamous epithelioma of cervix, involving bladder. Symptoms, six to eight months; hæmorrhage and fœtid discharge. There was a large crateriform ulcer on the cervix. Following an application of 1886 millicurie-hours in November, almost the entire surface had healed, leaving only a small area on the anterior lip, on January the 21st, 1925, when she returned for treatment.

Recurrent Epithelioma of the Cervical Glands.—M., aged 56. This patient was referred to me in 1921. An epithelioma, which developed in tuberculous scar tissue in the neck, had been excised four years previously. There was a tumour about the size of a walnut below the right submaxillary gland. This was first noticed about nine months previously, but latterly it had been growing rapidly. Following surface applications of radium in July and October, 1921, the tumour disappeared, but it recurred in October, 1923, when it again responded to treatment, but it reappeared in April, 1924. It was then about the size of a hen's egg, hard and adherent. Following three surface applications of radium (1700 mc.-hours) through 1 mm. lead, 2 cm. distance, the tumour again retrogressed, but it began to grow six months later, when it received 1400 mc.-hours through 2 mm. brass, 1·5 cm. distance. This patient is still under observation.

Sarcoma.—1. Prophylactic treatment was given to one patient after amputation of a finger.

2. M., aged 52. A small tumour was removed from the conjunctival surface of the upper eyelid in February, 1924, which on microscopic examination was found to be a sarcoma, and the growth recurred in April, when a surface application of 227·5 mc.-hours was given through ·5 mm. lead, 5 mm. distance. The tumour was scarcely recognisable in July, when 100 mc.-hours were given, with the same filter and distance as before. The growth disappeared, but it recurred in January, 1925. This patient is still under treatment.

3. Sarcoma of the mandible.—F., aged 14. A surface application of 2354 mc.-hours through 1 mm. lead, 3 cm. distance, was applied over the mandible; this was followed by 1600 mc.-hours under the chin, and again below the angle of the jaw. There was marked retrogression of the tumour within a month, and the trismus, which was a prominent symptom, had disappeared. In the hope of dealing effectively with possible metastases in the cervical glands,

"Deep Therapy" was given six weeks after the radium was applied. Following this the patient improved rapidly, and her facial expression became normal. Some months later she developed a cough, and an opacity in her thorax made one suspicious of a secondary deposit there. The latest report from her doctor is, however, reassuring, and she is doing well.

4. M., aged 73. Fibrosarcoma on dorsal surface of arm, which was excised in May, 1923. It recurred immediately, and an extensive removal of the triceps was made in June, 1923. In October, 1923, there was extensive recurrence, and amputation of the arm with the scapula was advised, but declined. Following surface applications of radium (2894 mc.-hours through 1 mm. lead) in October, 1923, the tumour disappeared almost completely by February, 1924. The supra-clavicular glands were enlarged and treated, and these nodules have been destroyed. A nodule which remained at the site of the original growth was again treated in February and July, 1924. When I saw this patient again in December there was apparently another recurrence in the posterior axillary fold. Needles were embedded in this, and also in the primary nodule, which had not completely disappeared. This patient is now having "Deep Therapy," and he is making good progress.

Carcinoma of Parotid Gland.—F., aged 65. A large excavated ulcer was freely discharging the glandular secretion, and there were metastases in the cervical and submaxillary glands. Following combined surface and embedded applications in May, there was a temporary improvement, and in September the ulcer was almost completely healed, but there were new secondary deposits in the neck. She was anæmic, and her powers of resistance were very feeble. Surface applications were again given, but she recently reported that she was doing badly.

Lymphoid growth in conjunctiva.—M., aged 31. There was protrusion of the mucous membrane around the globe. Conjunctival surface of upper lid was very granular. Symptoms, 15 months. In March he was given 54 mc.-hours through ·5 mm. lead and a thin layer of rubber. The plaque was laid on the cutaneous surface of the upper lid. There was considerable improvement in May, when 61 mc.-hours were given as before. When seen in June the granular condition of the conjunctiva had disappeared, and there was no puckering of the soft tissues surrounding the globe. Further treatment did not appear to be required, but the patient was advised to return for periodic inspection.

Carcinoma of Rectum.—M., aged 43. As this patient was treated in December, 1923, he should not technically be included in this review. A colostomy was done in May, 1923, as nothing else was feasible. He suffered much pain and discomfort from irritating discharge and tenesmus; he was cachectic and emaciated. Assisted by the surgeon who had performed the colostomy, I passed a 1 mm. brass tube containing radium emanation through the colostomy opening, but the stricture was impermeable, and I left it close to the tumour for twelve hours. Following this, an attempt to penetrate the stricture from below also failed, but I left the tube in close apposition with the neoplasm again from below. The following day, with the aid of a rectal speculum, I succeeded in passing the tube into the stricture, where I left it for six hours. The total radiation was 1140 mc.-hours. In July, 1924, this patient's local doctor wrote as follows:—"He has done remarkably well; in fact, he has put on a stone in weight. He is completely rid of his cachexia. The result in this case is certainly 'topping.'" On January 15th, 1925, the patient reports that he suffers no pain or inconvenience, and he is quite well and able to attend to his regular work as well as ever he did in his life.

As regards the technique of the treatment, the methods employed depend

on the site, extent, and general state of the lesion. If the body of the uterus is involved a brass tube containing the emanation is passed into the cavity of the uterus. If the os uteri is ulcerated the tube is placed in the cervical canal, and, if possible, needles are at the same time embedded in the os uteri. If the latter cannot be dilated needles only are implanted.

In all cases the vagina is tightly packed with gauze in order to keep the bladder and rectum as remote as possible from the radium, and also in order to keep the latter in a fixed position.

In no case has there been the development of rectal or vesical fistulae, or any other unfavourable complication.

My recent tendency has been toward the combined use of radium and high voltage X-rays in the treatment of inoperable oral and uterine carcinoma.

One application of radium will usually arrest the progress of the disease locally, and it will quickly promote the healing of an ulcer with negligible constitutional disturbance, and with little inconvenience to the patient, whose general health, as a rule, rapidly improves. Toxaemia is arrested, and the foetid discharge is diminished or altogether disappears, and no anaemia follows the treatment. Consequently it is possible very often to resort to "Deep X-ray Therapy" for the treatment of extensions into the parametrium, or metastases in the lymphatic glands, within three or four weeks after local treatment by radium, without waiting until cutaneous reactions have disappeared, or until the condition of the blood has been restored to normal.

Rontgen rays are better than radium for obtaining uniform radiation of large areas, and they should be employed to combat metastases as well as to obtain homogeneous distribution at depths which are inaccessible or dangerous for the implantation of radium. The effects of radium are for the most part local. What this means in its practical use is that only one-hundredth part of the radiation at the surface of a radium tube reaches a distance of 3 cms. The layers of tissue surrounding the tube receive an excessive radiation and rapidly necrose, and if the dose is excessive a slough forms, whereas the outlying cells two or three centimetres distant from the tube receive too little radiation. Radium does not afford such a homogeneous distribution of radiation over large areas as can be obtained with X-rays under ordinary conditions, and for this reason a combination of radium and X-rays is most desirable, particularly where a maximum local effect is desired in addition to the uniform irradiation of the surrounding tissues.

3.

The term "radium" is employed in its general sense, as since 1914, up to quite recently, I have used radium emanation in glass capillaries alone as my source of energy.

Since last March I have been utilising as well 53·93 mgs. of radium element in the form of sulphate. This is in small metal capillaries, and is employed in exactly the same way as the emanation capillaries, often in the same box or tube.

Malignant Cases.

Cancer of the Skin.—Nine cases treated, five were apparently cured. One of these had an epithelioma of the fore-arm for eight years and had been operated on several times. There was also a small gland at the elbow. When the ulcers had healed up and the epitheliomous mass had disappeared a small hard suspicious nodule was removed. This was proved microscopically to be only fibrous tissue. The small operation incision healed perfectly but was noticeably slow in doing so. One case with a large mass on the forehead

and glands under the ear was operated on three days after intense radiation of the fungating mass, leaving a clean, apparently healing ulcer. Subsequently there was some return of growth in this area, but the glands and the protuberance of the ear had disappeared. In another case on the back of the neck a large fungating mass disappeared leaving a healing sore. As this patient could not be operated on, on account of the condition of his heart, 24 radium emanation glass capillaries containing 46·5 mes. were buried in the tumour as part of his treatment and left there. As the tumour disappeared the capillaries worked out to the surface and were reinserted in slightly different positions. After six weeks they were discarded as useless. This dose may be reckoned as 6,200 mchs. Two patients with glands in the groin and axilla died from extension of the disease.

Cancer of the Lip.—Four cases. One apparently cured. One still under treatment. One died from exhaustion, palliative treatment having to be stopped on account of his critical condition. One with an enormous mass of glands returned to the country relieved of pain, and was lost sight of.

Cancer of the Mouth.—Forty cases.

Floor of the Mouth.—One case, improved at first. Lost sight of. Cancer of the upper alveolus, improved in an extraordinary manner after one treatment. Glands which had been removed six weeks before and returned, disappeared and the patient was completely relieved of pain; also the gum appeared normal after the treatment. I believe this patient died recently, possibly extension to the chest.

Tongue.—Thirteen cases. Five cases greatly improved when lost sight of. Three apparently cured. Three died: one after three years' treatment; one after eighteen months. In one case recurrent after operation, the glands disappeared, and the disease of the œsophagus became more manifest and was untreated. The glands in another case operated on two years previously disappeared, but the patient looked far from well when last seen.

Tongue and Tonsil.—Eleven cases. Six cases died: three of these were complicated by syphilis; two were under treatment for twelve and fourteen months respectively. Four cases were lost sight of, but were improved when last seen. One improved in a remarkable manner by a surface application alone, as he would not allow needles to be inserted into the tonsil.

Tonsil.—Three cases. Two of these, both elderly females, died: one, who did well locally, with signs of heart failure; and the other from septic absorption. One improved greatly at first, but was lost sight of.

Fauces.—One. This patient improved so much that he was apparently cured. Surface treatment was not applied in the first instance, and when he returned glands had developed, and he died with extension, but free from pain, after fifteen months' treatment.

Roof of the Mouth.—Two cases. One died of heart failure, and the second died about a year after the treatment with extension to the glands. Originally he had a mass which almost filled one side of his mouth, and it appeared remarkable how this mass disappeared and the area became covered by mucous membrane, and looked quite normal.

Upper Jaw.—Antrum. Four cases. One of these was fairly well nine months after treatment. Two were lost sight of. The last case was very much improved, but died about six months later from general debility. Local pain, scalding, and discharge had disappeared.

Naso Pharynx.—In this case the disease disappeared from the original situation, but recurred in the pharynx. Deep X-ray treatment apparently did not affect the new growth, and the patient died later.

Pharynx.—Two cases. Both were lost sight of.

Larynx.—Two cases. In one the symptoms were relieved, and six months afterwards I heard the patient was well, except that he had an apoplectic stroke. Another case in which an emergency tracheotomy had been performed improved and got fat after treatment. He seemed to be better after a subsequent treatment, but, I believe, died later from an intercurrent disease.

Oesophagus.—One was lost sight of. In a second the ulcer healed. He was given deep X-ray treatment, and died from bronchitis a couple of months afterwards. Another case was recent, and the fifth case received deep X-ray treatment subsequently, and died some time later.

Cancer of Rectum.—Five cases. One was much improved. A second, with disease of the colon as well, got relief of symptoms. Two in the last stages of disease died soon afterwards. One case, who had responded satisfactorily at first to deep X-ray treatment, was only temporarily benefited by one radium treatment, and died some months later.

Cancer of the Breast.—Nine cases. The ulcer in one case is apparently healing. A second was prophylactic after operation. A third has been lost sight of. In a fourth, three months after the operation, there was a recurrence in the scar and in the glands which caused œdema of the arm. This case was apparently cured, the œdema and mass completely disappearing. Two other cases were operated on before and after radiation, and have both done well. Another case, with a large fungating offensive mass and glands, has done very well with one treatment only. The breast and nipple regained their normal appearance, and a small residual thickened area gradually disappeared, without further treatment, as the patient recovered strength. Two patients died.

Cancer of Body of the Uterus.—This patient appeared to do extremely well, and said she was quite recovered six months after treatment.

Cancer of Cervix.—Three cases. One patient died sixteen months after the first treatment. The tumour in the abdomen appeared to increase markedly after one treatment with deep X-rays. She refused further X-ray treatment, and subsequent radium treatment had not much effect. Another patient died after eighteen months' treatment, the third being lost sight of.

Cancer of the Ovary.—One. This patient was treated for a rapidly growing mass one month after operation. She was too ill to leave her bed for deep X-ray therapy. External application of radium made the mass shrink to a remarkable degree, but it soon commenced to increase again, and she died after a couple of months.

Cancer of the Vulva.—One case. Marked temporary improvement. She has returned to the country, and I believe the disease has progressed lately.

Cancer of the Bladder.—All symptoms of hæmorrhage and pain disappeared for six months. Has not been seen since she was treated recently for slight recurrence of symptoms.

Cancer of Prostate.—Three cases. One felt better when he returned to the country, soon after treatment. A second had relief of symptoms, put on flesh, and appeared much healthier, but he has not been seen since he left hospital. In the third there was some relief of symptoms, and the tumour ceased to increase. He returned to the country. These cases were treated by radium tubes inserted through the urethra; in the rectum by surface application and by needles inserted through the perinæi. In no case was ulceration of the rectum produced.

Rodent Ulcer.—Ten cases. Four cases apparently cured; two lost sight of; four still under treatment.

Sarcoma.—Eight cases. One was radiated immediately after operation for a small growth, and is probably cured. Five were situated in the neck. One

of these patients, with a very large tumour, did very well. Two others were apparently cured. In one a large hæmorrhagic growth under the chin shrivelled up and disappeared. He would undoubtedly have bled to death but for emergency radium treatment, but unfortunately succumbed from extension to the lung some months later. Two in the thigh: one did very well, but has been lost sight of lately; another was very septic, and treatment had to be interrupted. He died before treatment could have had any effect.

In general terms, I may say my best results for some time have been obtained in cancer of the skin and of the breast. When a malignant condition attacks the skin and is localised, is should be possible to cure it by radium treatment if radium is any use in cancer; but the good results in cancer of the breast are more gratifying, as the condition is deeper seated and less accessible. In the latter part of this year I have been more satisfied with the results in oral disease by using distance pieces, and using up as much radiant energy as possible. I am forced to the conclusion that this method of "Deep Radium Therapy"—for want of a better name—is as important for final results as the treatment of the local condition. Undoubtedly here septic teeth and a foul condition of the mouth have a profound influence on the course of the disease.

Non-Malignant Cases.

Tuberculous Conditions.—Nineteen patients. *Lupus.*—One case of lupus erethematosus of the face was apparently cured. Another extensive lupus of the face is still under treatment, and has done very well. Ulcers and warty growths on the hand of a patient who has been under medical care for fourteen or fifteen years improved in an extraordinary manner with one to three mcs. of radium emanation in practically exhausted capillaries wrapped up in one layer of tea lead, which the patient put on herself in various areas for a week, according to direction.

Tuberculous Glands.—Three cases did very well. Two cured, one was lost sight of.

Tuberculous Abscess.—Four cases, two of which were local, were apparently cured, the other two were lost sight of.

Tuberculous Joint.—One tuberculous wrist in which the carpal bones were practically destroyed is making a good recovery. Radiation combined with inoculation and routine treatment undoubtedly helped this patient greatly.

Encephalitis.—Patient who was unconscious with brain symptoms was not relieved by radium treatment.

Lungs.—Three advanced cases. One of these patients improved temporarily, but left the hospital to live in a stable and could not be expected to recover. Two other patients appeared to be relieved while small doses of radium were applied for weeks at a time, that is their breathing was easier and their pain and discomfort less, and though the improvement was maintained for some time, both of them died later.

Tuberculous Peritonitis.—Four cases. One case was greatly improved by radium treatment. He left hospital when he was not fit to do so, and I believe died some months afterwards. Another case was lost sight of and probably died as the lungs were badly affected. A third case who was in very grave condition with lung symptoms and pleural effusion as well, made a complete and uneventful recovery. She received altogether 54 days' treatment. 58·9 mcs. were used during this time or a dose of 7,800 mchs. Another patient who was not in so grave a condition as the last received a dose of 40·9 mcs. or 6,700 mchs. during five days. His pain and discomfort were relieved, the swelling of

the abdomen decreased and he was able to go to the country about three weeks later. He subsequently had to take to his bed for six weeks in the country with pleurisy, but has recovered sufficiently to go to South Africa. He was going downhill when radium treatment was started.

Actinomycosis.—One case. After radium treatment this patient who had lockjaw was able to separate her gums about quarter inch, and I was able, under an anæsthetic, to completely open her mouth, which had been closed for over three months. The tumour of the cheek got smaller and she continued to improve when last seen.

Colitis.—One case, in the last stages of exhaustion from disease, was unable to benefit by the short treatment she received, and died a few days later.

Corn.—This corn, on the tip of the toe for six years, became very painful for 24 hours on the 22nd day after receiving a dose of 160 mchs. or 1·2 mcs. through 1 mm. brass (20 mcs. for 8 hours). The cornified skin with apparently the root attached, fell off without further trouble on the 42nd day after treatment, leaving behind perfectly normal-looking skin. The patient is now able to wear boots discarded for the last six years. This result appears remarkable as some years ago sections of skin tissue due to G.S. wounds, which I had treated with radium, were cut, and the epithelium and deeper lairs of the skin were thickened as if they had been increased by radiation. In these cases, however, I employed 3 mm. of brass and smaller doses.

Dupeytrin Contractions.—In three cases treated for recurrence after operation, fibrous nodules disappeared, the fibrous tissue softened, enabling the fingers to be stretched. Full erythema dose must be given to obtain these results.

Fibroids.—Two. In one case pain in a fibrous nodule in the breast has been relieved. A patient past the menopause, with fibroid of the uterus causing hæmorrhage, has apparently made a complete recovery by a dose of 26·3 mcs. or 3,500 mchs. in three localities in the uterus and cervix, and a 2,300 mgh. dose on the surface.

Fissures.—Two. One case of deep fissure of the lip of four years' standing, and one of the tongue, of some months' standing, have been completely cured by one application of radium.

Fractures.—In the case of Colles' fracture, the pain and stiffness and swelling were relieved by surface radium treatment, and undoubtedly the functional use of the hand was hastened.

Hodgkin's Disease.—One case. The glands in the neck practically disappeared very rapidly, almost immediately, on treatment, but have recurred to some extent again and will require further treatment.

Injury to the Elbow.—Pain and discomfort in one case have, I believe, been to a large extent relieved by radium treatment.

Keloid.—One case was apparently on the way to cure when last seen.

Lumbago.—Two cases were quickly relieved by surface radium treatment.

Nævus.—Four cases, including one of port wine mark, have improved somewhat by radium treatment, but will require extended treatment to obtain further improvement if the cosmetic results are to be good.

Neuralgia.—Two cases. Toothache was apparently relieved by application of radium on two occasions, and neuralgia of the tongue, which had prevented the enjoyment of food for several months and caused pain on talking, gradually and completely disappeared in the course of six weeks after radiation.

Osteo Arthritis.—Two cases. One case, patient who was only able to walk with difficulty on account of pain, stiffness, and swelling in the ankles, and who had lost the use of her hands on account of a similar condition of the

wrist, improved greatly with two applications of radium within a couple of months. She also received massage treatment, which, up to the time of radium treatment, had not been able to help her. She is now able to get about freely and do housekeeping. Another somewhat similar case, not nearly as bad, improved, but not so markedly as the first.

Pruritus Ani.—One case cured with one application of radium.

Rheumatism.—Knee. Relieved by one application temporarily.

Scars.—Fourteen cases. Pain in two appendix scars was relieved and tenderness disappeared. Two cases were radiated post-operatively to hasten return of function, and lost sight of. Nine cases were treated for G.S. wounds. Two of these were painful stumps of the upper arm, and are now able to use their artificial limbs, which they could not do previously. In another case whose brachial artery was tied, radium treatment in the scarred area has resulted in improved nutrition of the hand and the radial pulse can now be felt. It could not be found previous to treatment. Another patient with a painful scar of the hand was able to return to work at a foundry.

Sciatica.—One case. Immediate relief by treatment, undertaken when pain started.

Sinuses.—One chronic sinus healed. The other requires operative treatment.

Spring Catarrh.—One case cured, both eyes.

Tonsilitis.—Radium applied and patient felt all right next day. He stated that attacks usually lasted a week.

Ulcers.—Three. Quickly healed with small doses of radium.

Warts.—Three cases. Cured or lost sight of.

Hyper-Trichiasis.—Caused epilation, but hairs grew again, as further treatment not given.

4.

I have used the emanation chiefly in cases of superficial malignant disease and chronic inflammatory processes.

I have found its influence to be of the highest importance in the rare condition of malignant granuloma of the nose. This condition was first described by me some two or three years ago in the *British Medical Journal*. It was characterised by progressive ulceration and destruction of the tissues of the nose without the formation of a definite tumour. It was entirely resistent to everything in the way of treatment both medicinal and operative, but yielded like magic to the influence of radium.

Since then another case has appeared in Scotland under the care of a doctor who tried radium with, I understand, most gratifying results.

The only change introduced in the laboratory technique during the year was the introduction of a mechanical device for holding emanation capillaries during the process of sub-division. This eliminates handling during this stage of the work, and prevents the risk of injury to the operator's fingers.

The removal of the radium and the laboratory apparatus from Leinster House to Ballsbridge was effected without interrupting the supply of emanation. This was rendered possible by the kindness of Prof. Thrift in supplying liquid air, made in the Physical Laboratory, Trinity College, during the period while the Society's liquid air plant was being moved and set up at Ballsbridge. A temporary laboratory has been fitted up pending the building of the Society's new laboratories.

Early in the year the Committee were obliged with great regret to accept the retirement of Mr. E. A. Stone, who had taken an important part in the work of the Institute since its foundation. They feel sure that all users of emanation will join with them in wishing him many years of health and happiness to enjoy his well-earned leisure.

The Committee were fortunate enough to obtain the services of Dr. K. C. Bailey to fill the vacancy so caused.

E. COEY BIGGER,
Chairman, Irish Radium Committee.

No. 12.

A CONTRIBUTION TO THE GEOLOGY OF GREAT ORME'S HEAD.

By LOUIS B. SMYTH, M.A., Sc.D.,

Lecturer in Palaeontology in the University of Dublin.

(Plates III–VII.)

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Great Orme's Head is a promontory on the north coast of Carnarvonshire. A low isthmus, on which is situated the town of Llandudno, connects it with the mainland. The head is about two miles long by a mile wide, and reaches an altitude of 679 feet. The strata composing it belong entirely to the Lower Carboniferous, and are almost exclusively limestones, often dolomitised. A few thin shales occur, and there are sandy beds of very restricted extent.

At the eastern end of the head, overlooking Llandudno, the strata are tilted towards the north-west at about 30°, but over the greater part of its extent the dips are slight.

The present work is an attempt to make out the faunal succession in detail, and to correlate it with that of other areas. It will be presented in the following order:—

1. A brief general description of the succession.
2. Faunal lists, with notes on species.
3. Discussion of correlation.
4. Detailed account of exposures.

1.—Brief General Description of the Succession.

The highest beds on Great Orme's Head are those forming the prominence on which the summit is situated. They form a compact mass, having its centre approximately where the Great Orme Hotel stands, and measuring 1200 yards E. and W. and 500 yards N. and S. The thickness is estimated at 180 feet. They consist of thin, irregular beds of fine-grained, buff-coloured limestone, full of large straggling nodules of chert ranging from translucent dark grey and brown to opaque white. The general dip is to the N.W. at 10°–20°.

The beds immediately underlying the cherty series are well exposed at Bishop's Quarry. This is situated 300 yards S. of Great Orme Hotel, and is noted for the abundance and good preservation of its fossil shells. The beds have a general dip of about 10° to the N.W., and consist for the most part of dark grey limestones with several thin shale beds. A section is shown in fig. 2 (p. 153).

From the base of the dark Bishop's Quarry beds to the lowest bed which yielded identifiable fossils are about 710 feet of limestones, of which the upper 540 feet are mostly very light in colour, white to buff, and the lower 170 feet mostly dark brown. About 120 feet below the top of this series occurs a band with a large *Caninia*, which could be traced from the S.E. shoulder of the hill, round the western extremity to the N.W. corner. This will be referred to as the "*Caninia* Band."

The next easily traced horizon occurs about 160 feet lower, and consists of a group of nodular beds (see p. 154) of exceptional thickness, in places up to 10 feet, weathering to a red colour. As these beds give rise to the shelf upon which the lighthouse is situated, and as they contain no distinctive fossil, they will be referred to as the "lighthouse horizon."

The beds intervening between the Bishop's Quarry group and the lighthouse horizon are characterised by frequent bands of nodular limestone, often weathered to a red colour. They form the cliffs overhanging the Marine Drive from Porth yr Helyg round the western extremity to the Monks' Path.

Underlying the lighthouse horizon is about 260 feet of indistinctly-bedded white limestone, separated into two portions by a very coarsely nodular limestone. These two portions will be called the "upper" and "lower massive white limestones," respectively. They are well seen at Pen Trwyn, where the road is built in the nodular layer (Pl. V, fig. 1).

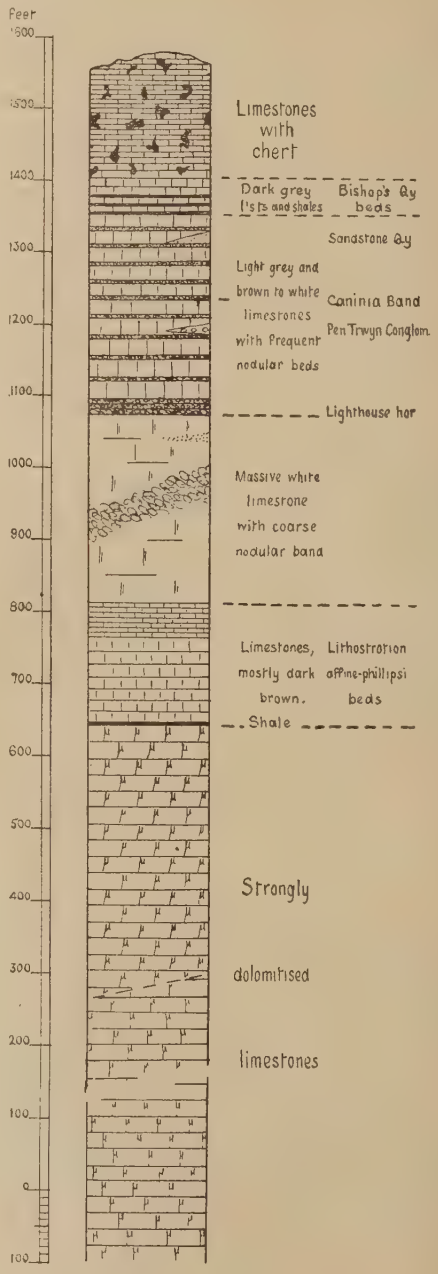

FIG. 1.

They rest upon thinly bedded limestones varying from light grey to dark brown, followed below by more thickly bedded, mostly dark brown, limestone. These predominantly brown beds, below the "massive white limestones," make up about 170 feet, and may be called the "*Lithostrotion affine-phillipsi* beds" from their characteristic fossil. At their base are a few thin shales, the first met with in descending below the Bishop's Quarry horizon, with the exception of a local bed in the sandstone quarry (see p. 159). The "*L. affine-phillipsi* beds" are conspicuous on either side of Happy Valley, where they have been quarried into cloister-like cavities.

Below the shales just referred to no identifiable fossils were obtained. The beds are mostly strongly dolomitised. Their thickness cannot be stated with any degree of assurance owing to interruptions of sequence which may be faults. Their base is nowhere exposed. In the extreme case there may be about 700 feet of these beds, but no more than about 300 can be counted upon. They are best exposed on the shore at the foot of Happy Valley.

The foregoing account is summarised in the vertical section, fig. 1.

2.—FAUNAL LISTS.

I.—*The Bishop's Quarry Beds.*

	Zaphrentis densa, Carr.
N	„ *enniskilleni,* E. and H.
N	„ cf. *oystermouthensis,* Vau.
	Caninia aff. *cornucopiae* (Mich.).
	Palaeosmilia murchisoni, E. and H.
	Lithostrotion maccoyanum, E. and H.
	Diphyphyllum lateseptatum, M'Coy.
N	*Dibunophyllum* cf. *vaughani,* Salée.
	Syringopora.
N	*Emmonsia parasitica* (Phil.).[1]
*	*Chonetes* cf. *elegans,* L. B. Smyth.
	Productus "x," abundant.
	„ *edelburgensis,* Phil.
	„ *giganteus* (Mart.), abundant.
	„ *hemisphericus,* Sow., and var.
	„ cf. *latissimus,* Sow. (near 3, Pl. XXXV, fig. 1.).
*	„ *margaritaceus,* Phil., abundant.
*	„ *pinguis,* Muir-Wood (MS.).[2]
	„ *productus* (Mart.).
*	„ *pugilis,* Phil.
	„ *sulcatus* Sow.[2]
*	„ *tissingtonensis,* Sibly, abundant.
N	„ *triquetrus,* Muir-Wood (MS.).
	Pustula aculeata (Mart.).
	„ *elegans* (M'Coy).

[1] Identified by Dr. Stanley Smith.

[2] Identified by Miss Muir-Wood.

	Pustula cf. *mesoloba* (Phil.).
N	„ *punctata* (Mart.).
	„ *spinulosa* (Sow.).
	Overtonia fimbriata (J. de C. Sow.).
N	*Schelwienella* cf. *crenistria* (Phil.).
	„ sp.
*	*Rhipidomella michelini* (L'Eveillé).
N	*Spirifer triangularis* (Mart.).
*	„ cf. *calcaratus,* M'Coy.
	„ sp.
N	*Spiriferina octoplicata* (Sow.).
*	*Athyris* sp.
*	*Reticularia* sp.
*	*Composita* aff. *ambigua* (Sow.).
	Pugnax sp. (near 3, Pl. XIX, fig. 10).
	Bellerophon cf. *costatus,* Sow.
	Posidonomya becheri (Bronn).
	Pinna flabelliformis, Mart.
	Phillipsia eichwaldi, Fischer.

* Species found in Bishop's Quarry, or its equivalent, loc. "OP" (p. 154, and map), and also at loc. "ON" (p. 154, and map).

N Species only obtained at loc. "ON."

Notes on species in list I.

Zaphrentis cf. *oystermouthensis* Vau. (Pl. IV, fig. 3). The younger stages agree rather closely with Vaughan's species (13, p. 553), but in the adult the septa become amplexoid. The counter septum retains its exaggerated thickening throughout.

Lithostrotion maccoyanum E. and H. agrees with the authors' description (1, p. 195). Average diameter of corallites 2·8 mm., largest about 3·5 mm.; number of major septa 10–11; length of minor septa = $\frac{3}{4}$ to $\frac{7}{8}$ that of major.

Chonetes cf. *elegans* L. B. Smyth is well represented by Davidson's fig. 12, Pl. XLVII (3). Ornament exactly as in *Ch. elegans* (21, p. 22), but it differs from that form in being auriculate.

Productus "x." A short, wide giganteid. Width 8–9 cm.; length = $\frac{1}{2}$ width. Form somewhat as in Davidson's fig. 3, Pl. XL (3), but "ears" more strongly differentiated. The "peculiar interruption and bifurcation of ribs," to which Davidson draws attention in his figure, occurs in some specimens. The costae are broad and flat. Close to the cardinal edge of the pedicle valve they become coarse, turn obliquely towards that edge, and give rise to a fringe of spines, as in Davidson's fig. 1, Pl. XXXIX (3). Prominent spines are scattered over the pedicle valve at about one per sq. cm. Concentric wrinkles irregular and very shallow. Brit. Mus. specimens B 23987 and 23989 (Morton Coll.) from "Upr. black limestone," Pentre Halkin, Holywell, are very close to this form, but have less sharply demarcated "ears."

Productus hemisphericus Sow., var. resembles in shape a very small *Pr. auritus* Phil.

Productus margaritaceus Phil.—In most specimens there is a spine at about the middle of the venter, and this is often one of two or three spines along the middle line of the shell.

Productus pugilis Phil. agrees very well with the form recorded by Garwood and Goodyear from "Upper D_2" of the Settle district (22, p. 204).

Compared with the form figured by Davidson (3, Pl. XLIII, fig. 2)—Brit. Mus. specimen B 13894—our form is larger, the costae somewhat finer and less distinct, semireticulation weaker and less regular, and trail longer.

Spirifer cf. *calcaratus* M'Coy is very like the form from "$D_{2\text{-}3}$ of Lolley Scar Quarry near Pateley Bridge (Yorkshire)," figured by Vaughan under the name *Sp. calcaratus* M'Coy (13, Pl. XLI, fig. 5c).

Athyris sp.—The outline is near Davidson's fig. 6, Pl. XVIII (3), but the shell is narrower in proportion and smaller, and the valve intersection is uniplanar. The length, 19 mm., of the pedicle valve is exactly equal to the breadth. Fringes ctenoid, with very narrow uncut proximal flange and broad teeth, 5 or 6 in 5 mm. There are specimens of this form from Great Orme's Head in the Wheelton Hind Coll., 1921, Brit. Mus., labelled (in Jan., 1925) "*Cleiothyridina royssii* L'Eveillé."

Composita aff. *ambigua* (Sow.) differs from the type chiefly in having a wider hinge, so that the slope of the shoulders is not uniform, but consists of an upper, less steep and a lower, steeper part. There are in the British Museum specimens of this form from Great Orme's Head (Wheelton Hind Coll., 1921); and from near Mold, Flintshire, said to be D_3; also of a closely similar form from "D_1 Redesdale, Northumberland."

II.—*Between the Base of the Bishop's Quarry Beds and the Base of the "Caninia Band."*

Caninia cf. *samsonensis* Salée.
Palaeosmilia aff. *murchisoni* E. and H.
Lithostrotion irregulare (Phil.).
„ *irregulare-martini*.
„ cf. *maccoyanum* E. and H.
„ cf. *portlocki* (Bronn).
Diphyphyllum cf. *lateseptatum* M'Coy.
Lophophyllum probably *magnificum* Nich. and Thom.
Dibunophyllum (*Histiophyllum*) sp. nov.
„ aff. *muirheadi* Thom. and Nich. (incl. *D. matlockense* Sibly).
Aulophyllum fungites (Flem.) mut. towards *pachyendothecum* (Thom.) S. Smith.
Syringopora.
Chaetetes depressus (Flem.).
„ (meandrine form) (25).
Productus derbiensis, Muir-Wood. (MS.).[1]
„ aff. *hemisphericus*, Sow.
„ *latissimus*, Sow.

[1] Identified by Miss Muir-Wood.

Productus cf. *maximus,* M'Coy.
„ *productus* (Mart.).
„ *scabriculus* (Mart.).
„ *striatus,* Fischer.
„ *triquetrus* Muir-Wood (MS.).[1]
Schelwienella sp.
Schizophoria sp.
Spirifer near *bisulcatus,* Sow.
Brachythyris ovalis (Phil.).
Athyris obtusa, M'Coy, abundant.
Cyrtina septosa (Phil.).
Psammodus rugosus Agass.
Psephodus magnus Agass.

Notes on species in list II.

Caninia cf. *samsonensis* Salée (15, p. 48) is the characteristic fossil of the "*Caninia* Band." Specimens have been kindly examined by Mr. H. P. Lewis, who writes as follows:—"In size, width of dissepimental zone, number of septa, and especially in the characteristic tendency for the minor septa, while projecting for a short distance within the theca, to be continuous across the inner dissepiments, and only partly continuous across the outer ones, the species . . . is identical with a Caninia collected by Professor Garwood in the D_2 beds of Humphrey Head, near Grange" (13a, p. 530). "It may differ from this species by having thickened denticulate bases to the septa, both major and minor, at the epitheca, and also by having fewer large dissepiments near the periphery.

"In most respects the species also resembles a specimen of *C. samsonensis,* Salée, which I possess, and which was collected in my presence from V 2 c (= D_2) beds of Samson, near Namur. In the Belgian species, however, there are only 60 major septa for a diameter of the corallum of about 7 cms., while in the Llandudno specimens, for a diameter of 6·5 cms., there are 70 major septa.

"For the present, until better preserved material is obtained for study, the species would best be referred to *Caninia* cf. *samsonensis* Salée."

Lithostrotion cf. *maccoyanum* E. and H. differs from the form in list I above in the greater number of septa (13–14 majors) and the shorter minors (= ½ length of majors).

Lithostrotion cf. *portlocki* (Bronn).—Average diameter of corallites, 7 mm. Number of major septa, 20–22. Three specimens, *a, b,* and *c,* showed the following differences:—

Diameter of theca in mm.	... *a,* 4·5	*b,* 4	*c,* 3
Ratio of length, $\frac{\text{minors}}{\text{majors}}$	... *a,* ½	*b,* ½	*c,* ¾

In most corallites of *b* and *c* majors reach columella, but in *a* none reach it.

[1] Identified by Miss Muir-Wood.

Diphyphyllum cf. *lateseptatum* M'Coy differs from the author's description of his type (2, p. 88) thus:—there may be up to five rows of vesicles in the wall; instead of branching dichotomously, four, or more, sub-equal branches are formed together, as in M'Coy's figure of his "*Cyathophyllum*" *parricida* (2, Pl. III c, fig. 9).

Dibunophyllum (*Histiophyllum*) sp. nov. (Pl. IV, fig. 1a, b) resembles *D. aspidiophylloides* Gar. and Gdyr. (22, p. 260, Pl. XVI, fig. 3) in its large central area, loose septal region, weak median plate, and marked fossula. It differs from that species in that the major septa are fewer (57, as against 65, for the same diameter), and are not specially thickened in the median area, and the minors do not reach the inner wall.

III.—*Between the Base of the* "Caninia *Band*" *and the* "*Lighthouse Horizon.*"

Lithostrotion junceum (Flem.).
„ cf. *portlocki* (Bronn).
Dibunophyllum.
Rhodophyllum sp. nov. (Pl. IV, fig. 2).
Aulophyllum fungites (Flem.) mut. towards *pachyendothecum* (Thom.) S. Smith.
Productus margaritaceus Phil.
„ *striatus* (Fischer).
„ spp.
Pustula cf. *mesoloba* (Phil.).
Overtonia fimbriata (Sow.).
Chonetes sp.
Athyris obtusa M'Coy.

Note on species in list III.

Lithostrotion cf. *portlocki* (Bronn).—The columella is absent in some transverse sections. The major septa average 23 in number, and do not reach the columella. Diameter of theca 5 mm. Average diameter of corallites 7·5 mm.

IV.—*The "Massive White Limestones."*

Palaeosmilia cf. *murchisoni* E. and H.
Lithostrotion irregulare (Phil.).
„ *junceum* (Flem.).
„ cf. *martini* E. and H.
„ cf. *maccoyanum* E. and H.
Dibunophyllum.
Syringopora.

V.—*The* Lithostrotion affine-phillipsi *Beds.*

Caninia juddi (Thom.).[1]
Palaeosmilia murchisoni E. and H.
Lithostrotion affine-phillipsi.
„ *junceum* (Flem.).
„ *martini* E. and H.
„ cf. *maccoyanum* E. and H.
Lophophyllum θ Vau. (Pl. IV, fig. 4a, b).
Dibunophyllum aff. *muirheadi* Thom. and Nich. (incl. *D. matlockense* Sibly).
Carcinophyllum vaughani Salée (Pl. III, fig. 5a, b).
Clisiophyllum delicatum sp. nov.
Lonsdalia duplicata (Mart.) (Pl. III, fig. 4a, b).
Syringopora ramulosa Goldf.
Chaetetes septosus (Flem.) (Figured 25, Pl. XV, fig. 3).
Productus cf. *maximus* M'Coy.
„ sp. nov.
Chonetes probably *siblyi* I. Thomas (= *compressa* Sibly).
Schizophoria.
Rhipidomella michelini (L'Eveillé).
Reticularia.
Dielasma sp. (cf. 3, Pl. I, fig. 14).
Tabulipora howsei (Nich.) Lee, var. nov.

Notes on species in list V.

Lithostrotion affine-phillipsi.—This descriptive term is here used to cover forms with the anastomosing habit described by Edwards and Haime for their *L. phillipsi*, but varying much in size of corallites. The average diameters of corallites in seven colonies were 8, 8, 9, 10½, 11, 13½, and 15½ mm., respectively. In the latter colony the range of size was from 11½ to 19 mm.

Lithostrotion cf. *maccoyanum* E. and H. differs from the authors' description (1, p. 195) in that the septa are more numerous (average number of majors 14, instead of 10–12) and the minors shorter (about ½ the length of the majors, instead of ¾ to ⅞). Transverse sections show many spaces, in contact with which the corallites have rounded outlines. The average diameter of corallites is 2½ mm. in a specimen from the base of these beds, 3½ mm. in one from near the top.

Dibunophyllum aff. *muirheadi* Thom. and Nich. (Pl. III, figs. 1, 2, and 3). Under this name I have included all the specimens of *Dibunophyllum* found at the base of the *Lithostrotion affine-phillipsi* beds, as well as some from higher horizons (see list II) although there is considerable variation.

The largest specimen obtained (spec. "k," fig. 1) has a diameter of 45 mm. at the calyx, decreasing to 34 mm. in a length of 75 mm., where the corallum is broken off. The epitheca is marked by fine transverse wrinkles superposed upon coarse irregular annulae. There is no longitudinal ornament.

In a transverse section (fig. 1a) 15 mm. below the floor of the calyx (diam. 45 mm.) the *central area* is oval, 11 × 15 mm.; the median plate reaches the margin of the area on the cardinal side, is thickened in the inner part of the

[1] Identified by Mr. H. P. Lewis.

area, and curves and comes to an end before attaining the margin on the opposite side; about 20 lamellae can be counted round the periphery, but only about half that number reach, or nearly reach, the median plate; tabellar intersections are crowded near the margin, sparse near the plate.

The *tabular region*, about 5 mm. in width, contains only 2–4 tabular intersections in each loculus, due to the rather large tabular vesicles (see longitudinal section, fig. 1c); its outer boundary has no stereoplasmic thickening.

The *external area* is about 10–11 mm. in width; the dissepiments are more crowded at its inner and outer margins.

The major *septa* number 69, are moderately thickened, taper slightly towards their inner ends, and are greatly attenuated and somewhat flexuous as they approach the epitheca, which they all reach; their inner ends are bent in a direction which, when viewed from the distal end of the corallum, is counter-clockwise. The cardinal septum is thinner and somewhat shorter than the rest. The minors are thin and flexuous, reaching from the epitheca inwards about $\frac{1}{4}$ to $\frac{1}{3}$ the width of the vesicular wall, and indicated the rest of the way to the theca by the "herring-bone" arrangement of the dissepiments.

In the most proximal transverse section obtained (fig. 1b), 34 mm. in diameter, the minors are much shorter in proportion, and the central area is more irregular, with 14 lamellae at its periphery, and the median plate short, unthickened, and confined to the interior.

In this specimen the wall of the calyx is broken away, its floor is rather flat, but rises slightly to form a tumid lip surrounding a deep slot-like depression with a pear-shaped outline. The broad end of the pear is central, and is just wide enough to accommodate the boss-like protuberance of the central column, which fails to rise to the level of the lip. The narrow end of the pear is 7 mm. deep, and reaches 12 mm. from the centre of the boss towards the cardinal septum.

This is the only specimen in which the calyx was exposed; consequently it cannot be said whether any of the other specimens to be described had a similar structure.

This specimen ("k") may be connected through specimen "d" (Pl. III, fig. 2) with a small one, specimen "μ" (Pl. III, fig. 3) which represents the bulk of the material from this level, and also is fairly complete. Taking the various transverse sections of these three specimens and plotting the number of major septa against the diameter of the theca (as being more constant than the total diameter) the points practically lie on a straight line.

The most mature transverse section of "μ" (fig. 3a) has a diameter of 20 mm. The description of the large specimen, "k" (fig. 1), would also apply to this, allowing for its smaller size, with these exceptions:—there is a trace of stereoplasmic thickening in the vicinity of the fossula; the cardinal septum is connected with the median plate by a thin straight prolongation. In the more proximal sections of this specimen (diam. 11 mm. and downwards) the theca is strongly thickened all round. In specimen "d" (fig. 2), referred to above, this thickening occurs at a diameter of 22 mm.

We may next consider specimen "e" (not figured). This seems to be sharply distinguished from those described above by its strongly developed minor septa. In a transverse section of 35 mm. diameter the minors of the cardinal quadrants show stout projections into the tabulate region, and are continuous from that to the epitheca. Corresponding to this feature, the dissepimental vesicles are rectangular in section. In the counter quadrants, although the minors do not enter the tabulate region, many of them traverse the whole vesicular wall, the rest varying down to half that length.

When, however, a section 15 mm. lower (diam. 30 mm.) is examined, the difference from the other specimens vanishes. Here no minors project beyond the vesicular wall, and only one can be found traversing its whole width. The minors of one cardinal quadrant, indeed, are extremely short. In all other respects the transverse and longitudinal sections agree with the specimen first described, i.e. fig. 1.

From a comparison of the specimens described, and many others from the same bed, it seems probable that we are dealing with a single species. The following points may be noted:—The median plate may be thickened, or not, and it may traverse the whole central area, or stop short of the periphery at the counter end, occasionally at both ends; stereoplasm is present in the proximal parts, but the stage to which it persists is variable; the minors may traverse the whole thickness of the vesicular wall and project into the tabulate region, or, on the other hand, may be mere teeth not reaching far from the epitheca.

Comparisons:—*Dibunophyllum* nr. *muirheadi* Thom. and Nich., S. Smith (12, Pl. XVI, figs. 2 and 3). S. Smith's fig. 2 agrees closely with specimen "k," fig. 1a, while his fig. 3 can be exactly matched amongst the smaller specimens from the same bed. Both are attributed to "Dy."

D. matlockense Sibly (9, p. 74, Pl. I, fig. 2), if not specifically identical with our form, is, at any rate, extremely close. Sibly says that his species shows "marked individual variation as regards the development of a secondary series of septa . . . The figured type has secondary septa well developed." We have seen that the Llandudno species may have better-developed minors than Sibly's figure, but mostly has them less well developed. *D. matlockense* is the predominant *Dibunophyllum* in D_2 of the midlands.

Our form also shows considerable resemblance in transverse section to *D. bristolense* Gar. and Gdyr., early form, as figured by Vaughan (13, Pl. XL, fig. 7, left), but does not agree in longitudinal section with that species as figured by Garwood and Goodyear (22, Pl. XVI, fig. 6b), the Llandudno form having a proportionally narrower central column and looser tabulate region.

Specimen "d," larger transverse section (fig. 2a), comes very close to Thomson and Nicholson's figure of *D. muirheadi* (5, Pl. XXV, fig. 4).

Clisiophyllum delicatum sp. nov. (Pl. IV, fig. 5a, b).—Conical or cornute at first, becoming cylindrical. Epitheca in earlier part with distinct interseptal ridges. Mature diameter 18 mm., surface of calicular boss making angle of 45° with axis; septal lamellae ascending its sides without any spiral twist. Rest of calyx unknown.

The features of the transverse and longitudinal sections will be seen in the figures. From many of the described species it differs in that the minor septa do not project into the tabulate area.

Comparisons:—*C. multiseptatum* Gar. (13a, p. 560) is larger, and the tabulae in the median area are not so vesicular. *C.* aff. *M'Coyanum* Thom., Sibly (9, p. 73), is larger, has a looser central area in which the lamellae are spirally curved, and the major septa are thickened in the tabulate area. *C. ingletonense* Vau. (12a, p. 251) differs in having a central area divisible into nucleus and aureole, tabulae in medial area less vesicular, and thickened major septa.

The type specimen, the one figured, will be deposited in the British Museum. It was collected from a bed of shale at the base of the *Lithostrotion affine-phillipsi* beds in the road cutting 300 yards W. of Pen-morfa Lodge on the S. side of Great Orme's Head, Llandudno.

Tabulipora howsei (Nich.) Lee, var. nov. agrees perfectly with Lee's description (14, p. 166), except in two points:—the number of apertures in a distance of

1 cm. is 30, not 40; the apertures in the diaphragms are oval (some a little irregular), not reniform.

Productus sp. nov.—Largest specimen 63 mm. wide. The form is rather like that of *Pr. corrugatus,* strongly arched antero-posteriorly, and with steep flanks. Concentric ribs very slight near cardinal margin; absent on venter. Radial ornament, like that of *Pr. hemisphericus,* consisting of fine, thread-like costae not alternating in strength.

Shell of pedicle valve thin on trail, but greatly thickened in visceral region, and especially just behind the brachial cavities, where the thickening forms a conspicuous ridge, with a roughened top. The cast consequently shows a sharp geniculation at the brachial impressions quite absent from the outer surface of the valve, which is evenly curved. The divaricator impressions are deep.

Davidson's fig. 2, Pl. XXXVI (3), would represent quite well the corresponding aspect of the ventral cast of this species, but, compared with the other figures of *Pr. "humerosus"* in the same plate, our shell has the adductor scars entirely posterior to the divaricator, and the casts of the brachial cavities shorter, blunter, and not sloping so much posteriorly.

3.—Correlation.

The Bishop's Quarry Beds.

The *Productus pugilis* occurring here appears to be the same as the form recorded by Garwood and Goodyear (22, pp. 204–5) from the Settle district, N. of the Craven faults, and regarded by them as "an important zonal index near the summit of D_2."

The fauna of "Upper D_2" ("Lamellibranch Beds") at Fairweather Springs, which includes this species, is in close agreement with the Bishop's Quarry assemblage. The same authors conclude that this horizon is "the equivalent of the lower portion of the Hardraw Shale, which . . . contains abundant examples of *Posidonomya becheri*" (loc. cit.), and in which, according to Hudson (24, p. 183), *Productus tissingtonensis* attains its maximum.

Correlation is therefore made with "Upper D_2" of Garwood and Goodyear.

A considerable number of the species are noted by Sibly from D_2 and D_3 of the Midlands, about half a dozen from D_3 only (9, pp. 44 and 50).

The Bishop's Quarry fauna seems also to be equivalent to the "$D_{2\text{-}3}$" assemblage recorded by Vaughan from Gower (13, p. 555).

Hind and Stobbs describe the "Aberdo" limestone occurring at Gorsedd, Holloway, Waenbrodlas, and Trelogan in North Flintshire (8, pp. 394–7). It consists of dark limestones and shales, overlies white limestone, underlies cherts, and is remarkable for the abundance of *Productus giganteus.* Probably, therefore, the Bishop's Quarry beds represent the same horizon.

The Lithostrotion affine-phillipsi *Beds.*

Carcinophyllum vaughani Salée (= *θ* Vaughan) appears to be confined to D_1 in the north of England (12, p. 605; 16, p. 26; 20, p. 122; 22, p. 213). In the S.W. Province its range is S_2–D_1, with a maximum in D_1 (7, Pl. XXVIII; 13, p. 548; 18, p. 145).

Lophophyllum θ (Vau.) is confined to D_1 in the Bristol district and in West Cumberland (7, Pl. XXVIII; 20, p. 122).

The range of *Lonsdaleia duplicata* (Mart.) is given by S. Smith (17, pp. 240–2) as D_2 and higher, but Edmonds records it from the White Beds of

the Fourth Limestone in West Cumberland, which lie beneath the *Girvanella* Bed taken as the base of D_2 (20, p. 129).

Caninia juddi Thom. has not hitherto been reported from a lower horizon than D_2 (23, p. 398).

Dibunophyllum matlockense Sibly is a D_2 form in the Midlands (9, p. 74), and *D.* aff. *muirheadi* Thom. and Nich. agrees with forms from the Dryburn and Fell Top Limestones, which are probably higher than D_2 (12, pp. 596, 598, 644; 13a, p. 547).

The remainder of the species in the list seem to be consistent with any horizon in D.

It seems probable that we are here at the base of the D_2 sub-zone.

Brown limestones preponderate in the *L. affine-phillipsi* beds. Since these come in under a great thickness of white limestones, it is natural to compare this horizon with the "Lower Brown" of Morton. An exposure of this is described by Hind and Stobbs at the base of Moel Hiraddug, S. of Dyserth (8, p. 391). The rock at this place, actually containing *Seminula* aff. *ficoidea*, was found to be identical with that in question at Great Orme's Head. But in the latter locality neither *Seminula* aff. *ficoidea* nor *Daviesiella* was discovered, and the fauna of the *L. affine-phillipsi* beds seems to indicate the top, rather than the bottom, of D_1.

In accordance with the above observations the following correlation is suggested:—

Bishop's Quarry beds = "Upper D_2" of Garwood and Goodyear, 1924 (22).
Lithostrotion affine-phillipsi beds = base of D_2.

The intermediate beds contain a fauna which is consistent with their position between these two levels.

4.—Detailed Account of Exposures.

The Cherty Series.

The largest exposure is in a cliff facing N.E. about 100 yards N. of Great Orme Hotel. The dip is 20° to the N.W. About 300 yards E. of this, close to the road to St. Tudno's Church, are some old artificial openings exposing similar beds dipping 10° to the N.W. A little S.E. along this road is a newer opening (quarry "ON") where these beds can be seen resting upon thicker, more regular limestones with chert and accompanied by purple shales. These underlying beds are fossiliferous and will be referred to later (p. 154).

Cherty beds can be seen in small excavations on the E. side of the summit, probably exploratory pits connected with the former copper mining. At the summit and southwards down the slope the beds, where seen, are coarsely dolomitic and dark brown or red.

On the golf links, about 150 yards in front of the club house, is a small quarry in cherty limestone dipping to the S. at 15°, and strongly dolomitised in patches. The upper limestones here are thin, irregular, and very cherty, the lower are in beds of from one to two feet thickness, have less chert, are purplish, and are interbedded with thin purple shales. The exposure resembles quarry "ON," referred to above, but no fossils could be found.

There is a small outlier 1000 yards E. of the summit and separated from the main mass by 500 yards (Pl. V, fig. 3). The occurrence of this is quite anomalous and will be dealt with later (p. 161). It may be noted here, how-

ever, that its beds are identical with those exposed on the N. side of the main mass. A specimen of *Lithostrotion junceum* was found here, the only fossil obtained in the cherty series, if we exclude those from quarry "ON."

The Bishop's Quarry Beds (fig. 2).

Bishop's Quarry has been worked on two faces looking N. and S.E. respectively. The topmost beds seen in the N. face are of light grey to brownish crinoidal limestone, containing abundant chert nodules. Here occur silicified specimens of *Lithostrotion maccoyanum*. Below this level chert becomes inconspicuous and, in fact, has only been seen in small and sparse blebs in the *Productus giganteus* beds.

Below the cherty top layers are about 20 feet of fine-grained grey to brown limestones with *Productus pugilis* and *Pr. "x."*[1] Near the base they assume a darker grey, and tend to break with a conchoidal fracture. These rest upon a bed of shale, about 1 foot thick, full of *Pr. margaritaceus*, and with plentiful *Pr. tissingtonensis*. The shale is dark grey, and the beautifully preserved, pearly white shells and white crinoid ossicles stand out in strong contrast. *Spirophyton* markings are abundant in this bed. The "*margaritaceus* bed" runs across about half-way up the N. face of the quarry. On the S.E. face it occurs near the top.

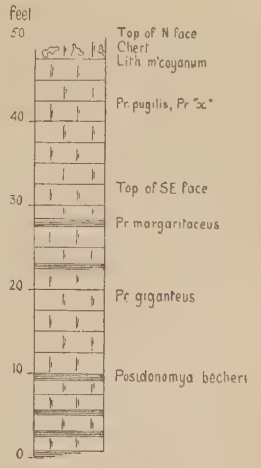

FIG. 2.

The beds next in sequence below are about 20 feet of fine-grained, dark grey limestone, with occasional flecks of chert, interrupted near the top by one thin bed of shale. This limestone contains towards its middle a surprising number of the shells of the typical *Pr. giganteus*, Mart.

From this to the bottom of the quarry are about 10 feet of alternating limestone and shale, both being dark grey. The uppermost of these shales, lying at the base of the *Pr. giganteus* beds, about 10 feet below its densest occurrence, contains *Posidonomya becheri*. Although the shale is only a few inches thick and exposed for a few feet along a vertical quarry face, several characteristic specimens were obtained.

The lowest bed visible in the quarry is a shale. One hundred yards to the east of this is a small escarpment of light buff limestone. Between this and the quarry the only exposures are a few small holes through the grass, revealing 2 feet of very dark grey limestone, with white crinoid stems, resting on the escarpment, and upon this a somewhat crinoidal bed weathered to a dark red. Between this and the shale at the base of the quarry there cannot be more than a few feet thickness of rock, the intervening ground being of the nature of a dip-slope littered with quarry debris.

From the small escarpment referred to, downwards for a great thickness, the beds are all of a much lighter colour (buff to white) than any in Bishop's Quarry, and will be described later (p. 160).

The S.E. face of the quarry curves round at its southern end until it runs E. and W. From this point the outcrop is hidden by sod, but, following the

[1] See p. 144.

probable outcrop towards the west, we come to a natural exposure of the same beds, running N.W. below the southern boundary wall of the golf links (loc. "OP"). It forms the brow of the slope which the Monks' Path ascends.[1] At the S.E. end of this exposure the uppermost bed or two contain chert nodules and silicified *Lith. maccoyanum*, as in the top beds of the quarry. Below these we can identify, in turn, the shelly *Pr. pugilis* beds, the *Pr. margaritaceus* shale, and the *Pr. giganteus* beds. The *Posidonomya* shale was not seen. The beds are all of a rather dark grey, and pass below into light-coloured limestones. At the N.W. end of the exposure the outcrop curves towards the N. and disappears under the golf links.

There are no other good exposures of these beds, but they can be recognised in various small openings. In a trench beside the road, 200 yards S.E. of the summit, and just E. of a small gravel pit (see O.S. 6-in. map), shelly beds are exposed, and *Pr. pugilis* was found. A few yards W. of the tram sheds and 550 yards E.S.E. from the summit they occur, lying horizontally in a shallow cutting and in an adjacent small pit. Just E. of the sheds a dark limestone and a bed of shale appear. On the N. side of the summit, about 200 yards N. of the hotel, and just inside the links wall, is a little excavation in shelly beds which may belong to this horizon.

Quarry "ON."

This quarry, 390 yards E. of the summit, already referred to (p. 152), may now be considered more fully. The upper part consists of the typical cherty beds, thin, irregular, and without fossils. These beds are here seen resting upon a fossiliferous purplish red shale, and below this are several feet of thickly-bedded, cherty, grey limestone, more or less stained by percolation from the overlying shale.

What is the relation of this limestone to the Bishop's Quarry beds? Amongst the fossils found here are *Productus margaritaceus, Pr. tissingtonensis, Pustula punctata, Choncles* cf. *elegans*, and *Rhipidomella michelini*. All these occur at Bishop's Quarry only in the "*margaritaceus* band," 20 feet below the cherty beds. Here, on the other hand, they immediately underlie the typical cherty beds, and are succeeded downwards by thicker beds containing chert. Other differences are the presence at "ON" of a red shale, the scarcity of *Pr. margaritaceus*, and the presence of the following fossils not found at Bishop's Quarry or at "OP":—*Dibunophyllum* cf. *vaughani, Zaphrentis enniskilleni, Z.* cf. *oystermouthensis, Emmonsia parasitica, Spiriferina octoplicata, Spirifer triangularis, Schelwienella* cf. *crenistria*. (Only a single specimen of each of the first five species was found.)

The most likely conclusion seems to be that the lower part of quarry "ON" exposes some beds not elsewhere seen, and belonging to an horizon in the lowest part of the cherty series, and just above the Bishop's Quarry beds. The only other possibility is their equivalence to the last-named beds. The fossils peculiar to "ON" have, accordingly, been included in the faunal list of the Bishop's Quarry horizon, but are distinguished by the letter "N" placed before them.

The Nodular Limestones.

A striking character of the cliffs of Great Orme's Head is the presence at frequent intervals of beds consisting, at the weathered outcrop, of rounded

[1] There is probably an unimportant fault at the copper lode at Ffynnon Gogarth, throwing down to the west.

nodules embedded in a soft, red, earthy matrix. Owing to their colour, and the fact that they generally weather back more rapidly than the other limestones, forming grooves, these beds form conspicuous features. They are confined to the light-coloured limestone series. The lowest one is a very thick bed between the upper and lower "massive white limestones"; the next is that at the "lighthouse horizon," also fairly thick; from the latter up to the base of the Bishop's Quarry beds they are thinner, 6 inches to 3 feet, and occur at frequent intervals, one of them being the "*Caninia* Band."

The nodules are rounded kernels of hard, light-coloured limestone, apparently quite similar to that in the ordinary limestone beds, sometimes stained a reddish brown. They are mostly somewhat ovoid in shape, but in certain beds, or parts of beds, a large, or sometimes even a preponderant, number of the nodules have a cylindrical, coral-like form.

Since the origin of these beds is under investigation, they will not be further discussed here.

The Western Cliffs.

On the S.W. face of Great Orme's Head is a cliff called Creigiau Cochion (the red crags), evidently from the conspicuous red nodular limestones described above. Here the strata have a nearly horizontal outcrop, and may be traced downwards from the beds of the Bishop's Quarry horizon on the escarpment above the Monks' Path (loc. "OP"). The "*Caninia* Band" is well exposed at an altitude of 540 feet. It is a red nodular layer, the large corals being on its upper surface, and it is immediately overlain by a limestone weathering into horizontal slabs.

Descending the cliff just below the cairn at the S.W. corner of the golf links, we find, 140 feet below the "*Caninia* Band," a bed, or series of beds, of red, nodular limestone, 6 to 7 feet thick, the "lighthouse horizon." This thick band runs beside the footpath and telegraph poles above the Marine Drive, and can be traced westwards to the road, which it crosses 120 yards S.E. of B.M. 403·1. It then appears below the road for a short distance, until cut out by the Hornby fault, which throws down 20 feet to the E. Rounding the western point of the headland, this band can be picked up again below the road to the east of the fault, and continues along the N.W. cliffs to the lighthouse, thrown down to the N.E. to a small extent by a fault at the Hiding Cave.

At the lighthouse point there are 160 feet of beds overlying the "lighthouse horizon." The horizon, therefore, of the topmost bed, on which the semaphore station is situated, should be about 20 feet above the "*Caninia* Band." This band was accordingly searched for and identified as one of the nodular layers at the brow of the cliff above the Marine Drive. On the N.E. side of the lighthouse spur its position can be indicated with the aid of a prominent mass of rock, separated by a cleft from the main mass above the road. The top of this mass is formed by a nodular red band. The next nodular red band above this is the "*Caninia* Band."

In the N.W. cliffs, between the most westerly point and the lighthouse, it is possible to examine a sequence of over 600 feet of nearly horizontal beds (fig. 3). The top, well seen at the semaphore, is 20 feet above the "*Caninia* Band," as already mentioned. From this to the "lighthouse horizon" are 160 feet of light-coloured limestone, with numerous thin nodular beds. The next 260 feet consist of the upper and lower "massive white limestones," separated by the lowest nodular limestone. The latter is a thick layer, with very coarse nodules, and with indefinite boundaries. It does not weather back to form a groove, as

do the thinner, less coarse, nodular layers met with so abundantly higher up, but stands in vertical walls even better than the massive limestone into which it merges. When freshly broken it is grey, in contrast to the white of the overlying and underlying beds. Cylindrical nodules are frequent. Immediately below this layer were found *Palaeosmilia murchisoni* and fasciculate *Lithostrotion.*

The lower "massive white limestone" rests upon the "*Lithostrotion affine - phillipsi* beds," a series of limestones about 160 feet thick, thin-bedded above, more thickly bedded below, and without any nodular beds. They vary from light grey to dark brown, the latter colour predominating. At the base is a thin band of very red earthy limestone, packed with *Lithostrotion martini* and *Syringopora ramulosa.* At the outcrop this bed has been weathered so

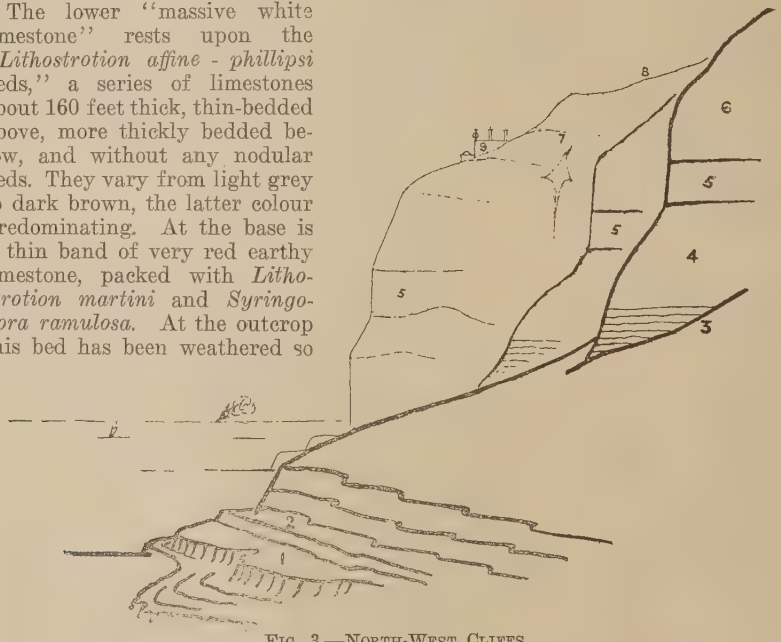

FIG. 3.—NORTH-WEST CLIFFS.

1. Shelf at base of *Lithostrotion affine-phillipsi* beds.
2. Coral bed.
3. Thin limestones forming top of *Lithostrotion affine-phillipsi* beds.
4. Lower massive white limestone.
5. Coarse nodular band.
6. Upper massive white limestone.
7. Nodular beds of lighthouse horizon.
8. Position of 400-foot contour.
9. Lighthouse.

as to leave the white corals standing out very beautifully in high relief against the red background.

Four feet below this, and a little above high water mark, follows a pronounced shelf, the upper surface of beds which disappear beneath the sea. These are very strongly dolomitised for the most part, but show towards the top some little-altered patches of brown limestone. The crystalline dolomite varies from red to yellow-brown. The upper surface of the shelf is composed of red shaly layers, with grey, coprolite-like, flattened nodules. The shelf is traceable from the most westerly point of the headland to the Hiding Cave. Here the fault already mentioned brings it below sea level.

The Northern Cliffs.

East of the lighthouse the coarse nodular layer, which occurs between the "massive white limestones," can be traced, sloping down to the east, until it

rests upon the rock platform which runs round the point immediately E. of Hafnant. This platform is therefore the lower massive white limestone, which is here at sea level. On its upper surface, and in the base of the coarse nodular layer, are corals, including *Palaeosmilia*, as at this horizon west of the lighthouse.

The shelf is succeeded by a small cove, at the east end of which, and N. of Dolfechan, is a small fault, which throws these beds down below sea level. They do not appear again until, more than half a mile to the east, the dip, which is here westerly, brings them up at the Pigeons' Cave.

The cliffs above the road from the lighthouse to Porth yr Helyg consist of the series between the "lighthouse horizon" and the Bishop's Quarry beds.

Pen Trwyn (Pl. V, fig. 1).

The rock shelf east of the Pigeons' Cave is formed by the lower "massive white limestone." The road round Pen Trwyn is constructed in the overlying nodular limestone. In this, a little to the S. of Pen Trwyn, by the roadside, can be seen a local unconformity (Pl. V, fig. 2). The vertical cliff above the road is the upper "massive white limestone," which gradually slopes down westwards, until it comes to lie below the road W. of Porth yr Helyg. Near the brow of this cliff is the "lighthouse horizon," forming a conspicuous red groove.

At the summit of Pen Trwyn, just below the cairn, about 70 feet above the "lighthouse horizon," is an interesting conglomerate bed. This rock consists of well-rounded pebbles of white limestone, just like that of the adjacent beds, in a matrix of the same material. The pebbles mostly range from the size of a gooseberry down to that of a pin's head. In the latter case the rock somewhat resembles coarse oolite. A few pebbles were observed up to 3 or 4 inches across. This bed is about 4 feet thick under the cairn, and extends about 150 yards along the outcrop, thinning out and becoming patchy until it merges into ordinary limestone.

The Happy Valley Section.

South of Pen Trwyn the lower "massive white limestone" can be seen ascending with the N.W. dip from sea level (Pl. V, fig. 1) until its outcrop, at an altitude of 270 feet, curves round the spur forming the N.E. boundary of Happy Valley.

This spur affords an extensive section. The lowest beds occur under the Grand Hotel, at the landward end of the pier, dipping at 30° to the N.W., and strongly dolomitised. An upward sequence can be followed for 200 yards along the shore, when a discontinuity occurs, which may be a fault. These beds are all dolomitic. Where the alteration is less extreme, e.g. 100 yards from the pier, abundant crinoid remains and corals can be seen. Their structure is too much injured for identification.

After the break similar beds are seen as far as the Rock Studio,[1] all coarsely dolomitic, with abundant crinoid remains. Immediately under the toll gate there is probably a slight fault.

Just N. of the Rock Studio the beds exposed on the shore become less dolomitic. They consist of dark grey, earthy limestone, with abundant *Spirophyton*, passing up through lighter shades to very pale grey. The *Spirophyton* beds contain *Lithostrotion affine* and *Productus* cf. *maximus*, and include a shaly layer with flattened cylindrical nodules, resembling those

[1] 190 yards N. of the toll gate, on the road.

described as occurring on the rock-shelf at the foot of the N.W. cliffs. This is the base of the *Lithostrotion affine-phillipsi* beds.

These beds are seen again above the roadway in an old quarry. On the south side of Happy Valley the same beds are easily identified under the eastern brow of Pen y Dinas, where the shaly bed with *Spirophyton* and flattened cylindrical nodules forms an extensive floor, and yielded *Prod.* cf. *maximus.*

Returning to the N. side of the valley, we find the beds in the quarry forming two large steps, each of which has been undermined by the workings so as to form a sort of cloister. The rock is chiefly rather dark grey to brown, becoming lighter above. These are the *Lith. affine-phillipsi* beds, already described as occurring in the N.W. cliffs, and their upper part here, as there, consists of thin-bedded limestones.

Resting on the latter is the lower "massive white limestone," which we have already traced up to this spur. From the base of this limestone downwards to the *Spirophyton* layer at the base of the *Lith. affine-phillipsi* beds is 170 feet, which agrees within a few feet with the thickness of beds in the N.W. cliffs intervening between the lower "massive white limestone" and the shelf with flattened cylindrical nodules near sea level.

The next 270 feet consist of the two "massive white limestones," separated by nodular limestone. There is a marked difference between the relative thicknesses of the two massive beds here and two miles away at the western extremity of Great Orme's Head. In the latter locality the upper band is the thicker, whilst here it is the thinner. The combined thickness of these two limestones and the nodular bed between is, however, about the same in both places.

In the middle of the upper "massive white limestone" the beds become very sandy, and are markedly wedge-bedded. The sand is a very pure quartz sand. This white calcareous sandstone weathers away, as is usual, more rapidly than the limestone, forming a groove in the outcrop. It can be traced from this spur as far as the roadway above the Pigeons' Cave. Similar material was found in an outcrop beside the road near Hafnant, at what must be about the same horizon.

The "lighthouse horizon" cuts the pathway on the N.E. side of Happy Valley at the 400-foot contour. From this to the crest of Craig Rofft are about 160 feet of beds, consisting of light-coloured limestone, with frequent nodular layers. These are the beds which form the greater part of the cliffs above the road on the N. side of the Head from Porth yr Helyg to the western extremity. The "*Caninia* Band" should be in the vicinity of the top of this set of beds, but could not be identified at Craig Rofft, or on the northern cliffs anywhere east of Hafnant.

At the crest of Craig Rofft there is a change of colour from the nearly white limestone prevalent below, the beds taking on a brown colour, but weathering to a thin white crust on the surface. The colour, however, becomes light again a little higher up. These beds are seen in the escarpment which rests on the dip slope at the top of Craig Rofft just S. of the words "Mynydd Isaf" on the 6-inch O.S. map (loc. "OJ"), in the cliff immediately N.W. of the same point (loc. "OH"), and in the prominence N.E. of it, which just reaches above the 400-foot contour. At "OH" there is a local unconformity, which will be referred to again (p. 159). Fossils are numerous here, notably *Productus latissimus* and *Spirifer bisulcatus.*

This horizon must be that of the beds immediately underlying Bishop's Quarry. This is evident in two ways. Firstly, the top of these beds is 235 feet above the "lighthouse horizon." The base of Bishop's Quarry is 120 feet above the "*Caninia* Band" in the S. section (see below, p. 160), and the latter

is 140 feet above the "lighthouse horizon" at Creigiau Cochion (see above, p. 155), making a total of 260 feet. Secondly, the outcrop of "OJ" can be traced S.W. to the sandstone quarry, to be mentioned presently (p. 159), near the tram sheds, where the dip is W.N.W. Immediately to the W. of the latter undoubted Bishop's Quarry beds are exposed, lying horizontally (see p. 154 above).

The dark beds of Craig Rofft reach to the edge of the cliff at Porth yr Helyg. Three small exposures around St. Tudno's cemetery show similar beds, probably of the same horizon. These are respectively 280 yards W., 100 yards S., and 100 yards E.N.E. of St. Tudno's church.

The Unconformity of Mynydd Isaf.

At "OH" (see p. 158 and Pl. VII) an unconformity can be seen in the face of the cliff, the dip of the beds below being about 5° in the line of outcrop, and the outcrop of the beds above being nearly horizontal. The lower beds are of a brown, compact limestone, with sparse crinoid ossicles and frequent shells. Resting on the plane of unconformity is a bed of purplish crinoidal limestone, full of rolled fragments of shell and well-rounded pebbles of limestone, often over an inch in diameter. The pebbles are partly of brown limestone, quite similar to the beds below the unconformity at this place, partly of white limestone. The rock is a fairly pure limestone, with no quartz sand.

The Sandstone Quarry.

Resting upon the topmost beds of Craig Rofft, E. of the tram sheds, is a quarry in sandstone. It is on the horizon of "OJ" and "OH," and just below the Bishop's Quarry horizon. Elsewhere on the hill this level is represented by limestones, with the possible exception of some slightly sandy limestones a little below Bishop's Quarry in the S. section (p. 160).

The sandstones in the quarry range from white to red or purple, and some beds contain quartz pebbles up to the size of a hazel nut. The beds are very inconstant. The lowest beds exposed in the quarry are about 4 feet of calcareous sandstone. Then follows a bed of quartz sand, cemented by red iron oxide, and quite devoid of carbonate. This grades upwards through 6 feet of calcareous sandstones to sandy limestone. The highest bed containing sand is a conspicuous thick bed of coarse white fragmental limestone, seen at the S. end of the quarry, consisting of rolled fragments of crinoids, etc., mingled with foraminifera and sparse grains and small pebbles of quartz. The contrast between the opaque rounded fragments and the transparent calcite cement is apparent to the naked eye. This bed can be traced to the S.W. along Pyllau Road to near the old mines. To the N.E. it thins out, and below it come in white and purple sandstones, with quartz pebbles. The fragmental limestone is strongly wedge-bedded, the wedges thickening northwards.

Resting upon it is a layer of compact limestone, about one foot thick, but tapering to nothing 100 yards to the north. This is a pale brown rock, breaking with a splintery fracture. In thin section, its grain is only revealed by the higher powers of the microscope. With low powers the general mass of the rock gives the impression of flocculence. There are islands of crystalline calcite, often representing the cavities of foraminifera. This rock, resting directly upon the coarse, false-bedded, fragmental limestone, strongly suggests calm after storm, for it could only have accumulated in perfectly still water.

Above this calcite mudstone is one foot of more normal limestone, followed by 6 inches of soft purple shale, and then a limestone with the same meandrine

Chaetetes which was found near the lighthouse (25). The horizon appears to be somewhat higher.

As already mentioned, there are small exposures of Bishop's Quarry beds immediately W. of the sandstone quarry, and evidently closely overlying it. They are lying horizontally, and must form the bottom of a slight synclinal trough, for the sandstones to the E. dip 10° W.N.W., and the Bishop's Quarry beds must, farther west, have an easterly dip to bring them up the slope, more than 100 feet, to Bishop's Quarry itself. This is confirmed by a small exposure half way up this slope which yielded *Productus pugilis*.

The South Section.

From the S.E. face of Bishop's Quarry a sequence of light-coloured limestones can be followed down the slope of the hill southwards to the sea-shore at Pen-morfa lodge. Down to the 400-foot contour the dip is 10° to the N.W., as in Bishop's Quarry, turning more to the W. as the outcrops are followed westwards. About 120 feet below the top is met the "*Caninia* Band," from which many large specimens of this coral, and some *Lithostrotion junceum*, were collected. Only a few inches of the top of this band are exposed above the grass. Immediately overlying it here, as at Creigiau Cochion, is a limestone which tends to weather into slabs parallel to the bedding.

Some of the limestones between Bishop's Quarry and the "*Caninia* Band" in this section were found to contain a small proportion of fine quartz sand. Three such beds were detected. In one of these some of the grains are complete quartz crystals, and crystal faces could be seen in a great many others. The grains from the other two beds do not exhibit this feature.

These sandy strata are at about the same horizon as the sandstone quarry half a mile to the N.E. (p. 159).

The nodular "lighthouse horizon" was not identified with certainty here. Indeed, in this section the nodular bands are not very prominent, for, owing to want of steepness in the upper part of the section, where they occur, they are generally overgrown, the intervening limestones forming escarpments projecting from the grass.

Between the 500-foot and 400-foot contours is a cliff formed by the "massive white limestones," which are much dolomitised in patches. At the base of the cliff is a grassy shoulder, 300 yards W. of the Sailors' Home. The edge of this shelf is formed by a prominent escarpment (OM 3, Pl. VII), which runs steeply down the slope to the sea in a S.W. direction. This and the beds immediately underlying it are well exposed in the road cutting E. of St. Petrock's, and on the pathway above it, and are those I have called the "*Lithostrotion affine-phillipsi* beds." Their base is exposed in a little quarry, "OM 1," on the pathway 230 yards N.W. of Pen-morfa Lodge. Here is seen a shale about 6 inches thick associated with brown limestones. It contains *Productus* cf. *maximus*, *Lithostrotion martini*, and abundant *Spirophyton* markings, and is evidently the shale horizon seen at the foot of Happy Valley (p. 157), and forming the shelf near sea level on the N.W. cliffs (p. 156). Calculating the thickness from this shale up to the "*Caninia* Band," we find it to be 590 feet, which agrees, within 10 feet, with the thickness calculated for the same series of beds at the last-mentioned localities,[1] thus confirming the correlation.

The shale of "OM1" quarry appears in the road cutting below, where it yielded a considerable number of corals.

[1] The brow of Craig Rofft is taken as the horizon of the "*Caninia* Band" at Happy Valley (see p. 158).

Below this shale no fossils were found. There are about 80 feet with recognisable bedding, and then all structure is lost in a crushed and highly dolomitised mass overhanging Pen-morfa Lodge.

Farther east the "*L. affine-phillipsi* beds" form the brow of the cliff above the Invalids' Walk, where a reef of that coral was found at the top of the zig-zag path. This escarpment is of pale grey limestone, passing laterally into the coarsest brown crystalline dolomite. Farther down the slope all exposures are strongly dolomitised. In several places near the zig-zag path weathering has brought out distinct wedge-bedding on a small scale in the dolomite.

Evidences of Local Erosion Summarised.

We may here usefully collect together the various cases where there are indications of current action. They are arranged below in their correct sequence from youngest to oldest:—

1. The sandstone quarry, pp. 159 and 160.
2. The unconformity at Mynydd Isaf, p. 159.
3. The Pen Trwyn pebble bed, p. 157.
4. Current-bedded, sandy layer in the "upper massive white limestone," p. 158.
5. Unconformity in coarse nodular band between massive white limestones, p. 157.
6. Wedge-bedding in dolomite, p. 161.

1 and 2 may be the same horizon. In 1 and 4 quartz sand is present, attaining in 1 the size of ½-inch pebbles. It seems legitimate to infer comparatively shallow water and proximity of land during the accumulation of the greater part, at any rate, of the Great Orme's Head sequence.

The Outlier of Cherty Limestone on Craig Rofft (Pl. V, fig. 3.).

The small outlier ("OS" Pl. VII) referred to when dealing with the cherty series (p. 152) is a bee-hive-shaped mound about 30 feet high and 100 feet in diameter. It rises abruptly from the dip-slope shelf above Craig Rofft at the head of Happy Valley, half-way between loc. "OJ" and the sandstone quarry. Its base is quite hidden by grass. The limestone at the top of the sandstone quarry can be traced passing to the W. of the knoll, that below the sandstones, and forming the crest of Craig Rofft escarpment, is continuous to the E. of the knoll. The knoll, then, if its position were normal, would be of the same horizon as the sandstones, from which it is separated by only 70 yards.

That it is out of its place is, however, obvious. It is too thick to fit in between the limestones immediately above and below the sandstone horizon. Its beds are, as already noticed, identical with those of the main cherty series, and no chert occurs elsewhere in the Great Orme rocks. The beds, moreover, are markedly tossed and twisted, although maintaining their general bedded arrangement.

There can be no doubt, in my opinion, that this curious hump is part of the cherty series. It is also clear that no arrangement of unconformity, or of faulting, can explain its present position, resting on an horizon below both the Bishop's Quarry beds and the sandstone quarry.

The tossed appearance of the beds suggests landslip. But the mound is practically on the 500-foot contour; the nearest point of the main mass of the cherty series is also at this altitude, 500 yards away; and the intervening

ground is concave. Obviously the amount of denudation since the escarpment of the main mass was in a position to shed the mound as a landslip is out of the question for post-glacial time, and we cannot imagine this small, steep-sided feature surviving the period of glaciation.

This leads naturally to another suggestion. May it not have been transported to its present position by the ice sheet? The Irish Sea ice is believed to have impinged on the N. Wales coast and there divided, one current turning to the S.W. across Anglesea, the other S.E. down the Welsh border. Great Orme's Head would be in the neighbourhood of the forking, and, in any case, must be on the debatable ground between the Irish Sea and Welsh sheets. Ice movements in almost any direction could be accounted for under such conditions. Striae have been observed on the S.E. slope of the headland, crossing one another obliquely, and having the directions N. 26° E. and E. 20° S. (6, p. 375). It is therefore quite possible that a mass might have been transported towards the east. Kendall and Bailey refer to such bodily transport of masses of beds, but on a larger scale, on the northern flanks of the Lammermuir Hills (11, p. 6). In the case of that at Kidlaw, "one-third of a mile long and a quarter broad," "the whole mass is in a completely shattered state."

Acknowledgments.

I have great pleasure in acknowledging my indebtedness to Miss Muir-Wood and Dr. Stanley Smith for much help with the brachiopods and corals, respectively; to Dr. F. A. Bather and Dr. W. D. Lang for giving me every facility in studying the British Museum collections; to Miss Goodyear, who, at great personal inconvenience, enabled me to examine N.W. Province material at Gower Street; and to Mr. H. P. Lewis for identifying the large Caninias.

Iveagh Geological Laboratory, Trinity College,
Dublin, *August*, 1925.

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EXPLANATION OF PLATES.

Plate III.

Corals from shale at base of *Lithostrotion affine-phillipsi* beds, 300 yards W. of Pen-morfa Lodge, Great Orme's Head. All figures one and a half times natural size.

Fig.

1. *Dibunophyllum* aff. *muirheadi* Thom. and Nich.; specimen "k" (p. 148); a, b, transverse sections; c, longitudinal section of one specimen.
2. *Dibunophyllum* aff. *muirheadi* Thom. and Nich.; specimen "d" (p. 149); a, b, transverse sections of a second specimen.
3. *Dibunophyllum* aff. *muirheadi* Thom. and Nich.; specimen "μ" (p. 149); a, transverse; b, longitudinal section of a third specimen.
4. *Lonsdaleia duplicata* (Mart.) (p. 148); a, transverse; b, longitudinal section of one specimen.
5. *Carcinophyllum vaughani* Salée (p. 148); a, transverse; b, longitudinal section of one specimen.

Plate IV.

Corals from Great Orme's Head. All figures one and a half times natural size.

1. *Dibunophyllum* (*Histiophyllum*) sp. nov. From 300 yards S. of lighthouse, just above "*Caninia* Band" (p. 147); a, transverse; b, longitudinal section of one specimen.
2. *Rhodophyllum* sp. nov. From 190 yards S.W. of lighthouse, "lighthouse horizon" (p. 147); a, transverse; b, longitudinal section of one specimen.
3. *Zaphrentis* cf. *oystermouthensis* Vau. From quarry (loc. "ON"), 390 yards E. of the summit (p. 144); a, b, c, three transverse sections of one specimen.
4. *Lophophyllum* (*Koninckophyllum*) *θ* (Vau.). From shale at base of *Lithostrotion affine-phillipsi* beds, 300 yards W. of Pen-morfa Lodge (p. 148); a, transverse; b, longitudinal section of one specimen.
5. *Clisiophyllum delicatum* sp. nov. Type specimen. From same loc. as fig. 4 (p. 150); a, transverse; b, longitudinal section of one specimen.

Plate V.

1. Pen Trwyn from the south. A, "lighthouse horizon"; B, road. Vertical cliff above road is the "upper massive white limestone"; that below road the "lower massive white limestone" (p. 157).
2. Local unconformity at Pen Trwyn. The photo represents a point on the roadside at about the centre of fig. 1, above. The hammer shows the scale (p. 157).
3. Mound of cherty limestone on Craig Rofft. The escarpment to the right is continuous with loc. "OJ" (p. 161).

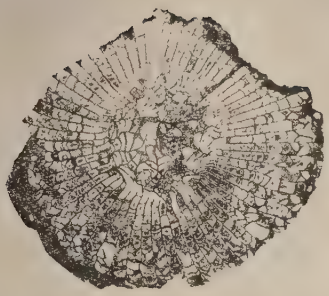
Plate VI.

Horizontal section through Great Orme's Head, along the line marked on the map, Pl. VII.

Plate VII.

Geological map of Great Orme's Head. Topography copied from O.S. 6-inch map.

1a



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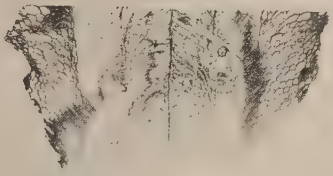

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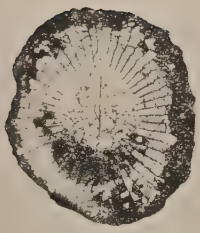
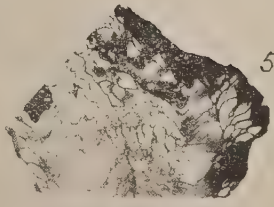


3a

2a

2b

3b



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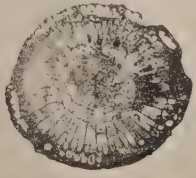
4a



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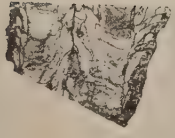

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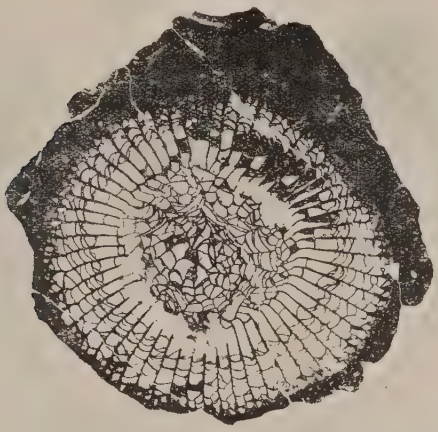

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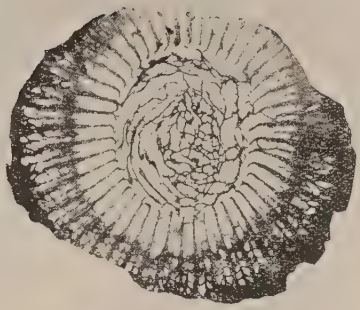

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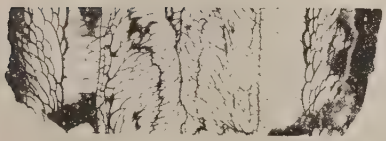

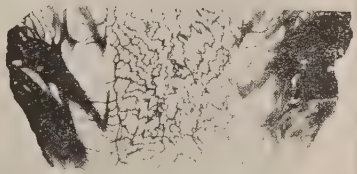

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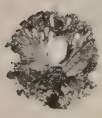

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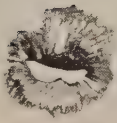

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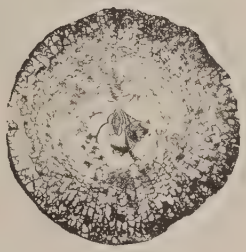

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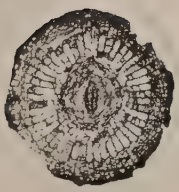

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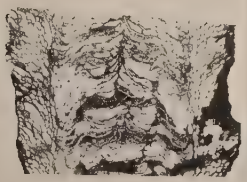


4b

FIG. 1.

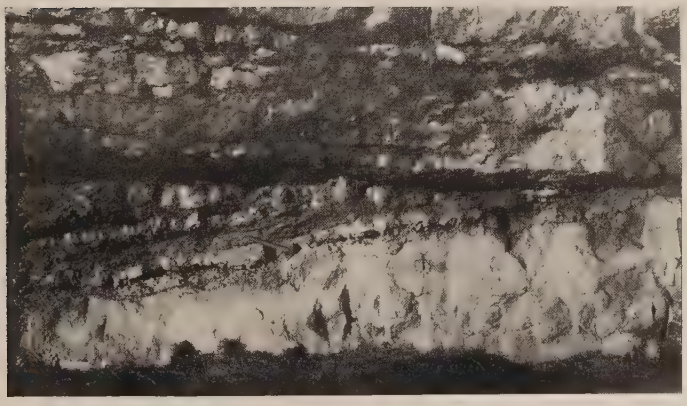
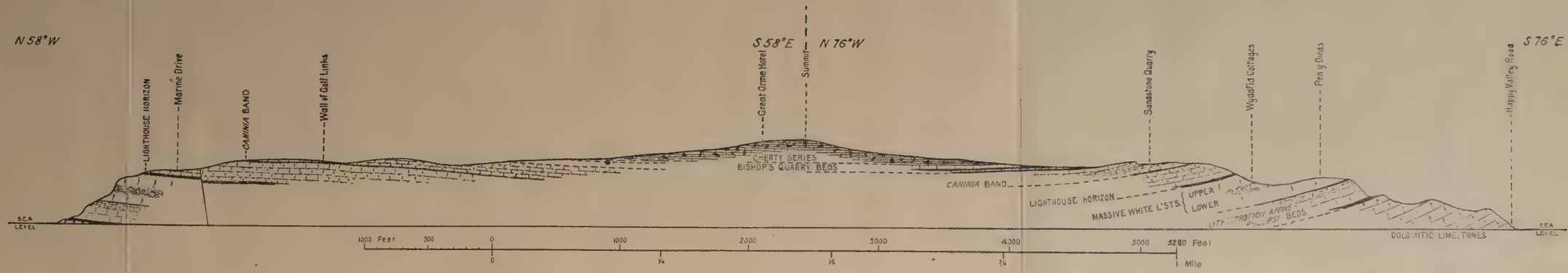


FIG. 2.

FIG. 3.



Horizontal Section through Great Orme's Head.

GREAT ORMES HEAD

No. 13.

THE ACTION OF RADON ON MIXTURES CONTAINING AMMONIA AND AN OXIDE OF CARBON.

By KENNETH C. BAILEY, M.A., Sc.D.,
Lecturer in Chemistry, Trinity College, Dublin.

[Read November 24. Printed December 1, 1925.]

Carbon monoxide and ammonia are capable of reacting in two ways, represented by the following equations:—

$$(1)\ CO + NH_3 = H_2O + HCN.$$
$$(2)\ CO + NH_3 = H_2 + HOCN.$$

Kuhlmann (Annalen, 1841, **38**, 62) considered that equation (1) represented the normal mode of reaction, and, by passing a mixture of the gases over thorium oxide, Mailhe and De Godon (Bull. Soc. Chim., 1920, iv, **27**, 737) succeeded in obtaining an excellent yield of ammonium cyanide (equation (1)). On the other hand, Jackson and Northall-Laurie (Trans. Chem. Soc., 1905, **87**, 433), by putting a mixture of these gases in contact with a heated platinum spiral, obtained ammonium cyanate and urea (10 gms. of the latter from 8 litres of carbon monoxide), these substances being clearly formed by the reaction of ammonia with cyanic acid (equation (2)). Some ammonium carbonate water, methane, nitrogen, and hydrogen were also formed, and only small quantities of cyanide. The investigators state that carbon monoxide was always in excess. If this means that the volume of carbon monoxide was greater than that of ammonia, the yield of urea, as calculated on ammonia, must have been almost quantitative. When the experiment was stopped at an early stage the main product was ammonium cyanate, which was subsequently converted into urea.

In view of the way in which the reaction may be directed so as to produce either hydrocyanic or cyanic acid to the virtual exclusion of the other, it seemed interesting to examine the action of radon on a mixture of carbon monoxide and ammonia.

Experiment A.—A mixture of 22 c.c.[1] of carbon monoxide and about 70 c.c. of ammonia was enclosed over mercury in a graduated burette. 90 millicuries of radon, derived from an aqueous solution of radium chloride, were sparked to remove electrolytic gas and added to the mixture, which was allowed to stand for thirteen days (10,860 millicurie-hours). A considerable contraction took place. The following volumes were recorded:—initial volume, 93·6 c.c.;

[1] All volumes are corrected to 0° C. and 760 mm. of mercury.

volumes after 1, 3, 7, 10, and 13 days—85·3, 76·9, 72·4, 70·4, and 68·5 c.c. respectively; total contraction, 25·1 c.c.

On addition of water 28·7 c.c. of insoluble gas remained. This was not analysed, but consisted, no doubt, of unchanged carbon monoxide, of hydrogen formed in accordance with equation (2) above, and of nitrogen and hydrogen formed by the decomposition of part of the ammonia, a decomposition known to be promoted by the radiations from radon (cf. Usher, Trans. Chem. Soc., 1910, **97**, 389; Wourtzel, C.R., 1914, **158**, 571).

The solution obtained (10 c.c.) was tested for cyanide, cyanate, and urea. The Prussian blue test for cyanides was carried out under carefully standardised conditions, so that the colour obtained could be compared with colours similarly obtained with solutions containing known amounts of sodium cyanide. As the test is rendered less delicate by the presence of ammonium salts, a portion of the solution was boiled gently with excess of sodium hydroxide to expel ammonia before applying the test. The amount of cyanide present was shown to be certainly less than 1 part in 20,000 of solution, and was probably nil.

The method proposed by E. A. Werner (Trans. Chem. Soc., 1923, **123**, 2577) was employed for the detection of ammonium cyanate. It depends on the formation of the sapphire-blue compound, $Cu(OCN)_2(C_5H_5N)_2$, which is soluble in chloroform. This test gave a fairly strong positive reaction.

The test for urea used was that proposed by Fosse (C.R., 1913, **157**, 948), depending on the formation of dixanthylurea when a solution of xanthhydrol in methyl alcohol is added to a mixture of glacial acetic acid and a solution of urea. The result was negative. When, however, the test was repeated with a fresh portion of the solution which had been heated in the steam-oven for five hours, a positive result was obtained. The presence of ammonium cyanate in the first instance (transformed into urea on warming) was thus confirmed. As the solubility of dixanthylurea in acetic acid—methyl alcohol—water is by no means negligible, urea may have been present in small quantity, and could possibly have been detected by adding an aqueous solution of xanthhydrol to a portion of the solution to be tested, acidified with hydrochloric acid, a modification of Fosse's test proposed by Werner ("The Chemistry of Urea," Edn. 1923, p. 178). This possibility was, unfortunately, overlooked till too late, and the difficulty of obtaining radon rendered a repetition of the experiment out of the question. The results, however, of experiments *B–D*, detailed in this communication, make it probable that urea was actually formed in small quantity.

We find, therefore, that radon radiations cause carbon monoxide and ammonia to react with formation of cyanic acid, no hydro-cyanic acid being formed (*i.e.* equation (2) to the exclusion of equation (1)).

Though one is, of course, unable to exclude completely the possibility of catalytic action (the material of the containing vessel may sometimes have an effect of this nature), it seems probable that the supply of energy to a mixture of carbon monoxide and ammonia, whether derived from a heated platinum spiral or an electrical discharge as in the experiments of Jackson and Northall-Laurie, or from the radiations of radon and its decomposition products as in the present case, promotes mainly the reaction,

$$CO + NH_3 = H_2 + HOCN.$$

A catalyst is necessary if the system is to react to any considerable extent in the way represented by the equation,

$$CO + NH_3 = H_2O + HCN.$$

Experiment B.—About 42 c.c. of carbon dioxide were placed over mercury in a burette, and ammonia was added till the burette contained about 100 c.c. of gas. The carbon dioxide and the first portion of ammonia admitted condensed on the surface of the glass as ammonium carbamate. 30 millicuries of radon were added, and four days later a further 15 millicuries. The experiment lasted 14 days (about 5,500 millicurie-hours). Water was added to dissolve the soluble substances present, and the solution tested for cyanate and urea. A small amount of cyanate and a trace of urea were shown to be formed. The experiment was repeated with a larger amount of radon in order to obtain a quantitative result.

Experiment C.—About 50 c.c. of carbon dioxide were passed into the burette over mercury in several small lots, and ammonia added each time, so that the carbamate was all condensed on a small area of the glass. Excess ammonia was finally added so that the initial volume of gas was 29·5 c.c. 100 millicuries of radon, freed by explosion from electrolytic gas, were added. A considerable expansion took place, the volumes of gas being 79·5 c.c., 97·5 c.c., and 108 c.c. after 7, 10, and 14 days respectively. The experiment terminated after 14 days (12,400 millicurie-hours). Water was then added and a small portion of the gas dissolved, together with the deposit on the walls of the burette, 95·6 c.c. of insoluble gas remaining (mainly nitrogen and hydrogen from decomposition of ammonia).

In none of these experiments could the writer detect any deposition of carbon.[1]

As the urea content of the solution was small, estimation was made nephelometrically. Standard solutions of urea were prepared, with concentrations ranging from 1 part of urea in 10,000 to 1 part in 100,000, a dilute solution of ammonium carbonate being employed as solvent in each case. 3 c.c. of each standard and 3 c.c. of the solution to be tested were acidified, the same volume of hydrochloric acid being added in each case. 20 c.c. of a saturated aqueous solution of xanthhydrol were added to each test-tube, and the contents were rapidly mixed. By noting the length of time necessary for the development of a perceptible cloudiness, and again by comparing the depths of opalescence after a certain length of time, it was possible to make a fairly accurate estimate of the urea content of the solution. The total quantity of urea present was 0·00125 gm.

Ammonium cyanate was also present and was estimated, but, as subsequent experiments have thrown doubt on the purity of the acetic acid used to neutralise the solution, the figures are withheld.

Experiment D.—Carbon dioxide was passed into a strong solution of ammonia till the latter was almost saturated. A solution was thus obtained which contained about 150 gms. per litre of ammonium carbonate and carbamate and a slight excess of ammonia.

10 c.c. of this solution were placed over mercury in a large inverted test-tube and 105 millicuries of radon were passed in. The solution was allowed to stand for 13 days (about 12,800 millicurie-hours). About 8 c.c. of insoluble gas were formed.

[1] Cameron and Ramsay (Trans. Chem. Soc., 1908, 93, 966) have noted that carbon is deposited when carbon monoxide or carbon dioxide alone is subjected to the action of radon.

Urea was estimated as before, the standards being prepared with the same strong solution of ammonium carbonate and carbamate as had been used for the experiment. The irradiated solution was shown to contain 0·00045 gm. of urea as well as ammonium cyanate, the figures for which were unreliable for the reason already given.

The small number of experiments performed and the unfortunate accident which deprived the cyanate estimations of their value make further work desirable. In view, however, of the fact that no radon is likely to be available for some time, the writer has decided to publish the results already obtained. Experiments on the action of ultra-violet light on similar mixtures are in progress.

The writer desires to express his thanks to Dr. H. H. Poole, of the Royal Dublin Society, for supplying him with the radon used in these experiments; to Mr. G. H. Deane for expert help in the manipulations involved; and to Mr. J. Bell, Sc.B., for some helpful and timely suggestions.

No. 14.

METHODS FOR INVESTIGATING THE VIRUS DISEASES OF THE POTATO, AND SOME RESULTS OBTAINED BY THEIR USE.

By PAUL A. MURPHY, Sc.D., M.R.I.A.,

AND

ROBERT M'KAY, A.R.C.Sc.I.

(Plates VIII, IX.)

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Introductory.

The present paper deals with certain experimental methods which have been evolved by the writers in the study of virus diseases of the potato, and with some of the results obtained by their use.

Atanasoff (2) has recently given a useful summary of methods of studying these diseases, with much of which we find ourselves in agreement; and, indeed, a technique similar to some there described for the first time had been developed independently by us. This is notably so in connection with the infection of tubers during the winter, and their subsequent planting in the open field. The success of such an experiment depends on the fact that the resulting plants develop symptoms of disease (usually secondary, but sometimes primary, passing into secondary) in the following June, that is, one month, and often much more, before the earliest appearance of primary symptoms resulting from the current season's infection. There is thus no danger of the two sources of infection being confused; and, when the control plant remains healthy, the source of disease is traceable unmistakably to the infection experiment carried out during the winter.

A requisite for success is a reliable method of tuber infection. It was as a result of the discovery made by one of us (6) that aphides feeding on the sprouts of potato tubers could act as infection agents that the method of planting in the open was first used in 1923 (8). The results were sometimes quite satisfactory; but, owing to the frequent unreliability of aphides in carrying infection, this procedure was subsequently used to a limited extent only. More recently it has come into favour with us again, owing to the discovery of an easy and reliable method of tuber grafting, which will now be described, and it was used with much success in 1925.

This procedure allows of a much greater volume of work than would be possible in an insect-proof greenhouse or in cages—these being the only satisfactory alternatives—and the expense is very much less. The method has its limitations, notably in that only one generation of plants can be grown, and it does not take the place of experiments begun in an insect-proof greenhouse and carried on for a second generation, which are absolutely essential in certain cases. Nevertheless, it is a valuable addition, and is much superior to infection experiments carried out in the open field, which indeed are too risky to be allowable under any circumstances in experimental disease plots.

Grafting Tubers by means of Cores.

Plane-grafting tubers.—One of the classical methods by which the infectious nature of leaf-roll was demonstrated (10) consisted of grafting the half of a diseased tuber to the half of a healthy tuber, as a consequence of which the shoots from the healthy portion eventually developed leaf-roll. These grafts were made by keeping the two plane surfaces of the cut tubers in as close contact as possible, by means of string, rubber bands, or other means; when, if organic union of the two portions took place, infection resulted; but not otherwise. The two non-grafted half-tubers served as controls, one to show that disease was present, and the other that the inoculated tuber was originally healthy.

Subsequently the same method was used with an amount of success which was generally partial and very rarely complete for this and other virus diseases. Among these may be mentioned mosaic (Quanjer, 11), mosaic (Schultz and Folsom, 13 and 15), leaf-roll (Schultz and Folsom, 14), leaf-roll (Kasai, 5), stipple-streak (Atanasoff, 1 and 3), "spindling tuber" (Schultz and Folsom, 15). This method was also used by the present authors for experiments (unpublished) on leaf-roll. The method of tuber grafting by contact of plane surfaces was successful in demonstrating the infectious nature of the above diseases in the generally small percentage of cases in which union took place, but as a reliable means of infection it was a failure. An exception must be made in the case of Atanasoff working with streak, but the generalisation that the method is equally applicable to other virus diseases does not appear to be justified.

Core-grafting and early experiments on disease transmission.—After an exasperatingly unsuccessful attempt to inoculate certain varieties with leaf-roll by "plane-grafting" tubers in 1923, experiments were set on foot in the autumn of that year to find, if possible, a better method. These were conducted at first without reference to any disease, and very soon a method was discovered by which organic union could be achieved with speed and certainty in 100 per cent. of the cases tried.

The procedure consisted in removing by means of a cork-borer of about 13 mm. diameter a core, containing an eye, from the tuber it was desired to inoculate, and inserting into the hole so made a core, removed with a cork-borer one size larger[1] than the one first used, from the tuber destined to serve as the source of infection (fig. 1, Pl. VIII). The latter core was taken without an eye at either end, and that from the healthy tuber (which contained an eye) was preserved for some time in a moist dish in the dark, and eventually served to raise a plant as a control.

When it was found that organic union was effected, a series of grafts was made, using diseased cores inserted into healthy tubers, in order to see if various diseases were transmitted with regularity. Attempts were being made at that time to analyse and synthesise certain combinations of mosaic diseases, and of mosaics and leaf-roll, and core-grafting was first made use of in this work.

The core-grafts shown in the following table were made between October 30th and November 10th, 1923. The grafted tubers were kept in a cool room during the winter, and were planted in 1924 in an insect-proof greenhouse or in muslin cages. Their progeny were in almost all cases grown for another season (1925) in the open field, and observations were made in both years as to the disease carried over, if any, and its identity. In certain cases the control core from

[1] If the tubers are shrunken, a borer two sizes larger may have to be used. A core of the proper size is a tight fit, but can be pushed into place by gentle pressure.

each grafted tuber was grown; in other cases, in which the healthy tubers had been raised in an insect-proof greenhouse devoted to the raising of healthy plants, one tuber representing each plant of the previous year served as a control. Since all the controls remained healthy, and the inoculated plants also appeared healthy at first, but developed symptoms of disease, in part primary and in part secondary, early in June, it is clear that infection is to be traced to the grafted cores.

Core Grafting in 1923–4.

Source of Disease.	Disease.	Varieties and number of each inoculated.	Total number of tubers which were grafted.	Total number of tubers which produced plants.	No. of plants infected.	Diseases and number of cases in which transmitted.
Irish Chieftain	Mosaic + streak	Irish Chieftain (2), President (3), Up-to-date (1), British Queen (1)	7	6	5	Mosaic + streak (4). Streak alone (1).
Irish Chieftain	Crinkle + streak	President (4), Up-to-date (1), British Queen (1)	6	4	4	Crinkle alone, possibly (2). Streak alone (2).
Up-to-date	Crinkle + streak	Irish Chieftain (1), British Queen (1)	2	1	0	——
Up-to-date	Mosaic + streak	Irish Chieftain (1), British Queen (1)	2	2	2	Mosaic + streak (1). Streak alone (1).
Irish Chieftain	"Curly dwarf"	President (4), Up-to-date (1), British Queen (1)	6	4	3	Mosaic alone, possibly (2). Streak alone (1).
Arran Chief	"Curly dwarf"	Irish Chieftain (1), Up-to-date (1), British Queen (1)	3	3	3	Mosaic alone, probably (1). Streak alone (2).
President	Streak	Irish Chieftain (1), Up-to-date (1), British Queen (1)	3	2	0	——
British Queen	Leaf-roll	Irish Chieftain (3), President (1), Up-to-date (1), British Queen (2)	7	7	0	——
Totals for diseases of mosaic group ...			29	22	17	
Totals for leaf-roll			7	7	0	

It may be seen from this table that of the 22 plants inoculated by means of a core-graft with diseases of the mosaic group, 17 became infected; while of the 7 inoculated similarly with leaf-roll, all remained healthy, as was evident both in 1924 and 1925. Taking the mosaic diseases individually, it appears that mosaic, crinkle, and streak were transmitted. The disease combinations were separated into their constituents in a number of cases, streak being isolated most frequently, as would be expected from its highly infectious nature. It is probable that mosaic was isolated in one or two cases, and, perhaps, crinkle in one. While 29 grafted tubers were planted, only 22 produced plants; and this rather large proportion of misses is related to the tendency of cores carrying streak and certain streak combinations to decay, and sometimes to induce decay or "blindness" in the grafted tuber. Further experience, however, has apparently eliminated the tendency to decay, which was connected with the use of vaseline to cover the ends of the cores.

Separation of mosaic diseases from leaf-roll by core-grafting.—It might be concluded from the first experiments that leaf-roll is never contracted by means of core-grafts. This is, indeed, generally so; but, as will be seen from the experiments now to be described, which were carried out in 1925 with a larger number of tubers, the disease is transmitted in a few cases.

In the first of these, the method of grafting by means of cores was tested by using as the source of disease tubers from plants containing a combination of leaf-roll and some unknown mosaic disease (or diseases), which grew in the plots in 1924, the object being to see if the mosaic disease could be separated from leaf-roll. The variety inoculated was Arran Victory, and, as the plants derived from control cores from every tuber showed, the stock was entirely healthy to begin with.

The grafting, which was done on October 31st, 1924, was carried out in different ways, as follows:—One lot of tubers was grafted while immersed in water which had been boiled and allowed to cool, and another lot was grafted in air, no water being used. Both lots were then divided in half, 6 tubers of each having the ends of the cores they contained covered with grafting wax,[1] while the cores in the others were left unprotected. All the tubers were then left in an unheated store until February 24th, 1925.

On that date it was decided to try the effect of incubation at 20° C., as advocated by Atanasoff (2), for tuber infections. Accordingly each lot was again divided into two strictly comparable portions, one of which was kept (with the corresponding control cores) at 20° C. for six weeks, while the other was left in the cool store as before.

All the tubers and cores were planted in the open field in April, and all produced plants. In following this method of planting (Atanasoff, *loc. cit.*) tubers infected during the winter (as originally recommended (8) by the present authors), it is very important that infection should be clearly visible well before there is time for primary infection of the current season's origin to appear (under our conditions, that is, before mid-July). In the following table, therefore, the time is shown at which infections were first noted, in addition to the method of grafting and the way in which the tubers were stored.

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[1] The ingredients of the grafting wax are: beeswax, 4 parts; resin, 2 parts; tallow, 1 part.

Core Grafting in 1924–5.

Source of infection, ... Three plants of Up-to-date diseased with leaf-roll and mosaic (or mosaics).

Grafted tubers, ... Healthy Arran Victory.

Method of grafting.	How stored for 6 weeks before planting.	No. of plants Inoculated.	No. of plants Infected.	Disease transmitted and date when first noted. 3/6/25.	10/6/25.	25/6/25.	7/7/25.
Under water. Grafting wax used.	At 20° C.	3	3	Streak.			Mosaic.
					Streak.		
				Streak.			
	In cool store.	3	3	Streak.			
				Streak.			
				Streak.			
Under water. No grafting wax used.	At 20° C.	3	3	Streak.		"Curly dwarf."	
				Streak.	Mosaic.	"Curly dwarf."	
				Streak.			
	In cool store.	3	3	Streak.			
				Streak.			
				Streak.			
In air. Grafting wax used.	At 20° C.	3	3	Streak.		Mosaic.	
				Streak.			
				Streak.			
	In cool store.	3	3	Streak.			
				Streak.			
				Streak.	Mosaic.		
In air. No grafting wax used.	At 20° C.	3	2	(Healthy)			
				Streak.			
				Streak.			
	In cool store.	3	3	Streak.			
					Mosaic.		
						Mosaic.	

The condition of the control plants, which were 24 in number, and were derived from cores, is not shown, but all were healthy.

It will be seen from the table that the 24 grafted tubers all produced plants, and that 23 of the latter showed one or more diseases of the mosaic group very early in the season. It should be noted that there can be no certainty that every tuber which provided cores contained the same mosaic disease combination. On the other hand they all contained leaf-roll, but this disease was transmitted in no single case. Compare in this connection figs. 2, 3, and 4, Pl. VIII, which show the separation of mosaic from leaf-roll in another experiment.

It may also be concluded that grafting under water possesses no advantage over grafting in air, but it seems desirable, though not absolutely necessary, to cover the ends of the cores with grafting wax. No conclusive result was apparent, either, from keeping the grafted tubers at 20° C. for 6 weeks previous to planting — infection with streak, at least, was not made quicker or more certain, but it is possible infection with other mosaic diseases may have been facilitated to some extent.

Further core- and plane-grafts, using impure leaf-roll. — Another entirely similar experiment was carried out simultaneously on 12 tubers each of British Queen and Arran Victory, but using another source of disease—namely, British Queen diseased with leaf-roll which had been grown in the disease plots since 1921, and in which, therefore, a good deal of mosaic infection might be presumed.

The result bore out that of the last experiment. The 24 grafted tubers all produced plants, and of these, 15 developed mosaic early in the season, while leaf-roll was transmitted in a single case. The 24 control plants (derived from cores) remained healthy. Grafting in air produced as good results as grafting under water; and keeping the tubers at 20° C. for 6 weeks before planting proved to be of no advantage.

A third experiment, also carried out simultaneously with the last two, and involving the same material as was used in them, demonstrated the advantage of core-grafting over plane-grafting. Six tubers of the variety Arran Victory were used, and although very carefully tied to half-tubers from diseased plants, the margins of the cut surfaces being covered with grafting wax, organic union appeared to follow in only three instances, and a mosaic disease was conveyed to two only of the corresponding plants. Leaf-roll was transmitted in no case. It is apparent that this method of grafting is not reliable, while it has the disadvantages of being more difficult, slower, and more wasteful of material.

Core-grafting with various mosaic diseases.—In the course of other experiments on various mosaic diseases, the method of core-grafting was employed on 24 tubers of a number of varieties, which were afterwards planted in the field or in cages. Definite infection resulted in 19 cases, and in one the result was doubtful. Control plants, derived from cores, remained healthy. Some of these grafts were made in the previous autumn, and some as late as February, 1925, but infection appeared to result practically equally early in all cases. These grafts were made, as a rule, using water and wax, and afterwards the tubers were stored in a cold room.

Conditions affecting transmission of leaf-roll by core-grafts.—When, in the earlier experiments, no leaf-roll infection whatever resulted from core-grafting, attempts were made to find an explanation for this. All the grafts had been made on a uniform plan, using a core without an eye which was inserted in a median position at right angles to the longitudinal axis of the healthy tuber (fig. 1, Pl. VIII). The failure might conceivably have resulted, therefore, from the virus being concentrated near the eyes, or from the core being situated rather far from most of the eyes of the healthy tuber. Grafts were, accordingly, made in other ways, cores from leaf-roll diseased tubers, with and without eyes, being inserted parallel to the longitudinal axis and at right angles to it. The eyes of diseased cores which contained them were cut out just before planting. The grafts were made in October, 1924, and the tubers were planted in an insect-proof greenhouse in 1925, together with the control cores. Of the eight grafted tubers, 7 produced entirely healthy plants—as did all the control cores—and one a plant diseased with leaf-roll. As it happened, the tuber responsible for this plant was grafted with a core containing an eye which ran through the longitudinal axis (incidentally, the diseased shoots were proved not to originate from the core), but this was clearly a coincidence. For, in another experiment carried out at the same time, leaf-roll was conveyed by core-grafting in one case out of four, and in this case the core was without an eye and was inserted at right angles to the longitudinal axis. In all the experiments, which

include 71 core-grafts with leaf-roll material, leaf-roll has been transferred five times, and in four of these cases the core contained no eye and was placed at right angles to the longitudinal axis of the healthy tuber.

On the other hand, there appears to be clear evidence that keeping the grafted tubers at 20° C. for some time before planting is actually unfavourable to infection with leaf-roll. This was most clearly shown in an experiment in which 4 healthy British Queen tubers were grafted with cores from tubers of the same variety diseased with leaf-roll on February 23rd, 1925. The cores were without eyes, and were inserted at right angles to the longitudinal axis. Two of the tubers (and their controls) were immediately put in an incubator at 20° C., and the other two, with their controls, were put in an unheated room. This treatment continued for 45 days, when all were planted, with the result that all the controls produced healthy plants, as did the two tubers kept at 20° C., but the two kept in the cool room. produced plants which showed secondary leaf-roll by June 25th.

Leaf-roll followed from core-grafting in five cases only, as has already been stated. It is significant that these instances occurred in four experiments in which half the grafted tubers were kept at 20° C., and half in a cold room, and that it was always tubers kept in the cold which produced the diseased plants.

It may be concluded that the rarity with which infection with leaf-roll by means of core-grafts takes place is an expression of the relative non-infectiousness of the disease, compared with diseases of the mosaic group. The latter diseases also differ among themselves in the ease with which infection takes place, and some evidence has been presented to show that streak, the most infectious of them, is most frequently conveyed. It may be assumed that the remaining diseases are transferred by core-grafts with an ease which is in direct proportion to their infectiousness.

Advantages and applications of core-grafting.—The question of core-grafting has been dealt with at some length because the method has proved so useful in our hands, and is one which is bound to meet general adoption, because it is simple, economical of material, comparatively certain in action, and can be carried out in winter. With simple precautions against the infestation of the sprouts by aphides, it does not lend itself to chance infections from outside sources, and the control core, being taken at the moment of infection and kept under the same conditions as the grafted tuber, is almost as perfect a check on the result as could be desired. These cores give rise to quite good plants, even under field conditions, when, however, planting by hand with a "dibble" after the drills are made up is recommended.

The method does not differ in principle from other methods of grafting, by using which disease combinations may sometimes be analysed, but its advantage lies rather in the greater certainty of its results, as is obvious in the separation of mosaic diseases from leaf-roll. The mosaic diseases themselves are also sometimes segregated by its use, and experiments are now in progress to see if by modifying the procedure they cannot be separated from each other with greater regularity. Any advance in this direction would greatly simplify the problem of diagnosing and recognising these diseases.

Core-grafting has been employed successfully by us in testing tubers destined, if proved free from disease, to provide healthy foundation stock for general propagation. The only point in question in this work is, of course, the presence or absence of mosaic diseases. The method was checked by top-grafting, and the results were in entire agreement, so that it may be recommended for this purpose, with the proviso that if every tuber cannot be tested

a sufficient number of grafts must be made from each plant-unit (a minimum of six might be suggested), and the whole unit must be discarded if any infection results. It is also useful for determining whether leaf-roll is combined with any mosaic disease (see figs. 2, 3, and 4, Pl. VIII), and for proving the health of tubers, particularly concerning freedom from streak, for experimental purposes.

Recent experience has indicated that when a virus disease is inoculated into a plant of another variety, it is advisable in many cases to transfer the disease back again to the original variety in order to prove that the same virus is still present without addition, or, as might readily happen if the first virus were a complex, without subtraction. This is particularly necessary when dealing with diseases of the mosaic group, the symptoms of which vary so much. It is also essential that all the inoculated plants should be grown until secondary symptoms of disease appear.

According to the best present methods of work this procedure requires at least two years to carry out, and entails the necessity for keeping the plants free from insects in the first year. Experiments are now in progress to endeavour to compress the whole operation into one season, and to make it unnecessary to grow the plants under insect-proof conditions at any time. It is hoped that this can be done by means of a core-graft made during the same winter from a tuber originally infected by means of a core from a diseased tuber; or, alternatively, by a sprout-graft following a core-graft.

It may be stated that in making core-grafts under water, we have evidence from one experiment that failure to change the water when dealing with different diseases does not introduce any complications. In all the remaining experiments, however, fresh water was used for each disease, and in all cases the cork-borers were sterilised before making a second graft by being dipped in alcohol, which was then burned off.

Other Methods of Infecting Tubers and Sprouts.

Sprout-grafts.—Grafting tubers by means of contact of plane surfaces has already been referred to as being unsatisfactory. Where, as in the case of leaf-roll, core-grafting is not successful, resort may be had to cleft-grafts carried out on the sprouts. This method is also applicable to the mosaic diseases, and in addition to leaf-roll, mosaic and streak have been successfully transmitted by us in this way. The greatest percentage of successes has been secured with streak; but, in general, there have been many failures owing to decay of the scions, and the method is besides slow and rather difficult, although very economical of material. The grafts in question were made on March 25th, and the tubers were then kept for six days at room temperature and afterwards at 20° C. for three weeks, when they were planted. Arran Comrade—a very susceptible variety—showed evidence of streak infection at that time.

Aphides.—Evidence has already been presented by us (9) to show that aphides feeding on the sprouts are not reliable infection agents, and that many variations in the conditions failed to produce more satisfactory results. Part of the difficulty, no doubt, is due to the fact that most of the work was done with leaf-roll, perhaps the least infectious of all the virus diseases, but uneven results have also been secured with the mosaic group. It may be stated, however, that all the diseases have been transmitted in this way at times, and that the following aphides were proved to act as carriers:—*Myzus persicae, M. pseudosolani,* and *Macrosiphum solanifolii.*

The following further experiments carried out in 1925 may be shortly summarised as being typical of what frequently happens. Twenty-nine halves or thirds of tubers in three experiments were infested with aphides from diseased sprouts. If one neglects the eight tubers of one experiment on which the aphides developed insufficiently, there remain twenty-one tubers, only two of which became infected (with leaf-roll, the aphides being *Myzus pseudosolani*). In these experiments the following factors were made variable—time on source of disease and on healthy tuber, temperature, and number of aphides—but none of these seems to have any bearing on the result. In the two instances of successful infection the aphides were fifteen days on the source of disease, and fifteen and twenty days respectively on the healthy tuber.

Experiments with nymphs.—Atanasoff (2) describes a method by which healthy aphides have been secured at times from parents whose condition of health was unknown, namely, by removing nymphs from parthenogenetic females, which were situated on potato sprouts, before the former had time to feed. A modification of this procedure has also been used independently by us, with similar results so far as our experience has gone. In the spring of 1923 seven healthy half-tubers were infested with nymphs from females feeding on diseased sprouts. Nymphs to the numbers of 7, 1, 1, 2, 38, 10, and 17 respectively survived on the half-tubers (as a rule there was a mortality of about 50 per cent.), and these were allowed to feed for some weeks, but in no case was any infection carried over. Again in 1924, two lots of non-pathogenic nymphs were raised in the same way. Experiments are at present being conducted with nymphs from females feeding on the foliage.

The method always used to secure the nymphs seems to be an improvement on that of Atanasoff. A number of mature females were first picked off into a glass, and from that were transferred one by one to another glass, each being examined with a lens to ensure that no nymphs, which are sometimes entangled with them, were carried over at the same time. The mature females were then kept enclosed in the glass for three to five days, during which time nymphs were produced in considerable numbers, and were transferred daily to potato sprouts. The method provides an absolute guarantee that the nymphs have not fed on diseased material.

Infection through Foliage and Stalks.

Shoot-grafts.—An easy and favourite method of infection is to graft a small diseased shoot, usually 3 inches or less in length, near the top of the shoot of a healthy plant. In our work cleft-grafts are always used, and these are bound tightly with fibre, no grafting wax being used. If the plant is in a greenhouse, it is then put below the bench for a week or ten days; but successful grafts have also been made in cages without protection.

The percentage of successful grafts reaches 100 with scions which are healthy or are diseased with leaf-roll, and usually so with those diseased with mosaic, but grafting with streak is more difficult, though not impossible, as we have successfully done it several times. In such cases, cutting the scion hard back, or using a scion provided with a bud only, but no foliage, is helpful; as is also close covering of the plant after the operation.

The advantages of shoot-grafting are that infection with every virus disease, and with every constituent of a combination, is made certain, if the scion lives for a sufficient length of time; and that infection becomes visible within a short period. If the top of the healthy plant is grown as a cutting (fig. 6, Pl. IX), the control so provided is almost as perfect as could be devised, but it should

be noted that if the detached shoot were in an early stage of infection the effect of forcing the shoot to form roots is frequently to delay the appearance of symptoms until the following year. (See experiments on rate of spread of virus in plant.) Second generation plants should, therefore, be grown.

The disadvantages of the method are that it requires expensive equipment in the way of greenhouses or cages, and that it is rather a drastic and unnatural procedure.

"Leaf-mutilation."—The "leaf-mutilation" method for transmitting mosaic diseases devised by Schultz and Folsom (12, 13, 15, 16) is better in the latter respect, because the amount of infecting material used is less, but the condition in which the plants are left is not favourable for the recognition of symptoms, unless a second generation is grown. We have used this method very little, but streak has been transferred by it with complete success, apparently, in the case of caged plants. One application of crushed foliage to the healthy leaves was made on July 10th, but no symptoms became visible during the first season. The method had previously been used unsuccessfully to separate a combination of leaf-roll and mosaic, but with further experience it may be found useful in this class of work.

Aphides.—Butler (4) has rightly emphasised the point that valuable information should follow from further study of infections carried out with the natural agents of dispersal—namely, insects. The consensus of opinion is that aphides[1] are the principal carriers, and, indeed, as many people believe, the only ones, although the latter conclusion is one which we, in company with others, have not been able to share. The difficulty, however, of using these insects in routine experiments to infect plants through the foliage is considerable, for apart from the question of bulky and expensive equipment, and the many possibilities of error, we have found aphides most unreliable as disease carriers.

Using aphides on caged plants in 1922, there was complete failure to transmit leaf-roll, the infested plants and their progeny remaining quite healthy in 1922 and 1923. The same results were obtained in more extensive experiments in 1924–25, for no infection was carried over, with the exception of one plant out of the 159 plants of the second generation. In some instances the number of aphides which survived on the healthy plant were counted some days after the infestation, and the majority were found to be alive and feeding. In another series the healthy plants were fumigated after 20 days, and here again there was a good development on all the 9 plants. In a few instances, however, the aphides did not settle down properly. The total number of plants infested with aphides in 1924 was 18, but the aphides were purposely allowed to feed on some of these for a short time, and in some other experiments their stay on the diseased plants was purposely varied. The general conditions were good, and were apparently favourable for the spread of disease, so that the only possible conclusion, seeing that these insects have at times conveyed infection, is, apparently, that some necessary condition was lacking. It is a safe deduction,

[1] The following have been identified as being implicated in carrying one or more of the virus diseases of potato:—*Macrosiphum solanifolii*, Ashm. (12, 9), *Myzus persicae*, Sulz. (12, 6), *Aphis abbreviata*, Patch (16), and *Myzus pseudosolani*, Theob. (9). Atanasoff has pointed out that some of the evidence on which the transmission of virus diseases of potato by aphides is based is not conclusive. The exclusive participation of these insects is not proved when diseased and healthy plants are caged together in the open and aphides are allowed to disperse from one to the other; or even when aphides are transferred in bulk, as on a leaf. Nevertheless, a critical study of the literature seems to leave no room for doubt that aphides can carry infection.

however, that negative results with any particular insects do not prove that they cannot act as carriers.

Capsids.—The possibility of employing other insect carriers remains, in which connection we have some further proof that capsids (*Calocoris bipunctatus*) sometimes transmit leaf-roll. In an earlier experiment in 1921, a plant infested with these insects, taken from plants diseased with leaf-roll, produced an entirely diseased crop in the following year. A half-tuber control, grown in an adjacent cage, produced an entirely healthy crop, as did also neighbouring healthy, but non-infested, caged plants to the number of more than twelve. The plants were not, however, under regular observation in the latter part of the season,[2] and to that extent the experiment was not entirely satisfactory.

An opportunity, which was being looked for, occurred to repeat this work in 1923, when capsids (from *Urtica dioica*) were placed on caged plants diseased with leaf-roll, and then transferred to caged healthy plants which were provided with half-tuber controls. Two of the infested plants were grown throughout in an insect-proof greenhouse, in which no green flies ever occurred, and minute special examination failed to reveal a trace of aphides on them at any time. Furthermore, there had been no leaf-roll in this house during that season, and before transferring to another house (in which again no aphides ever occurred) for the purpose of infestation, the plants were caged with fine "lawn" material. The cages remained in position till the end of the season. The third plant was grown in a cage outside.

The result of the experiment was that one of the two plants in the greenhouse developed primary leaf-roll 16 days after the infestation, and produced an entirely diseased crop in the following year (fig. 8, Pl. IX). The other two plants, and all the controls, remained healthy, as did all their representatives in 1924. This case of successful infection involved an unusually quick appearance of primary leaf-roll after the infestation; but if leaf-roll was not contracted through the agency of capsids, then the carriers of infection are entirely unknown.

EXPERIMENTS TO DETERMINE RATE OF SPREAD OF LEAF-ROLL VIRUS IN PLANT.

A method has been devised, which works very successfully, to study more accurately than has yet been done the rate of spread of a virus in the potato plant. This consists of growing a healthy plant with a single stem until a height of about 30 cm. is reached. The effect of allowing only one stem to develop is to force the axillary buds into growth, with the result that 8 to 10, or so, lateral shoots develop along the length of the stem. The main shoot is then grafted near the top with a diseased scion, and at appropriate intervals afterwards the lateral shoots are cut away close to the stem, and are grown as cuttings (compare fig. 5, Pl. VIII). Some of these may show disease in the current season, but the full results are secured only when their tubers are planted. The top of the main shoot, cut off in the operation of grafting, is also grown as a cutting and serves as a control (fig. 6, Pl. IX). Extra control plants derived from one-half of the tuber which produced the experimental plant have also been used, but a control taken at the moment of infection is preferable.

A modification of the experiment is to grow a single-stemmed plant and graft it as described, and then to remove the tubers at intervals. A tuber taken at the time of grafting makes a good control.

[2] We are under an obligation to Dr. G. H. Pethybridge for some notes on these plants during this period.

A number of experiments on these lines were carried out in 1924–25, leaf-roll being the disease used. The plants were grown in an insect-proof greenhouse in 1924, and the resulting tubers were then planted in the field, where the final results were secured early in the following June.

The next table shows the result of removing the lateral shoots at intervals from two British Queen plants, originally healthy, but grafted at the top with leaf-roll material on June 2nd, 1925. The lateral shoots were taken from one plant from the bottom upwards, and in the other from the top downwards. All the shoots are numbered from the bottom.

Shoots taken at intervals, beginning with the lowest.				Shoots taken at intervals, beginning with the highest.			
No. of shoot.	Days elapsing between grafting and removal.	No. of tubers planted.	No. and condition of resulting plants.	No. of shoot.	Days elapsing between grafting and removal.	No. of tubers planted.	No. and condition of resulting plants.
1	5	1	1 Healthy.	8	5	1	1 Healthy.
2	10	2	2 „	7	10	2	2 „
3	15	2	2 Leaf-roll.	6	15	1	1 Leaf-roll.
4	21	1	1 „	5	21	2	2 „
5	25	1	1 „	4	25	1	1 „
6	30	2	2 „	3	30	0	——
7	35	2	2 „	2	35	2	2 „
8	39	1	1 „	1	39	3	3 „

It may be seen that when the shoots were removed 5 and 10 days, respectively, after grafting they escaped infection, and this independently of their position on the main stem. After 15 days, however, all the remaining shoots were already infected. See fig. 7, Pl. IX. It may be concluded from this that it took the virus something longer than 10 days to travel from the scion to the third shoot from the top, but that within 15 days it had gone at least as far as the third shoot from the bottom.

A similar conclusion was reached from another experiment in which two healthy single-stemmed British Queen plants were grafted at the top with leaf-roll at the same time, the shoots (to the number of 7) from one being removed together after 6 days, and from the other after 15 days. As the plants produced by tubers from the cuttings in 1925 showed, infection had not reached any of the 7 cuttings removed after 6 days, but it had penetrated equally to all the 7 removed after 15 days.

An experiment dealing with the rapidity of spread of infection to the tubers, carried out on similar lines, showed that it took something more than 8 days and less than 14 days for the leaf-roll virus to travel from a scion situated 29 cm. above ground level to the tubers. The tuber taken at the time of grafting and that taken 8 days afterwards produced healthy plants, but the tubers taken after 14 days, and all taken afterwards (up to 49 days), produced plants diseased with leaf-roll.

The conclusion is clear that within 14 to 15 days the whole of the stem, including lateral branches and tubers, becomes infected with leaf-roll from a diseased scion grafted at the top. Since no infection is contracted within 10 days, at least, the diffusion through the stem is probably rapid. Experiments are now in progress to determine its exact rate, if possible.

It remains for future work also to show to what extent, if any, diffusion of the virus is hindered while union between stock and scion is taking place. Until this question is more fully investigated, it is not possible to apply these results to the interval elapsing between foliage and tuber infection under natural conditions. Unfortunately, two experiments to determine the rate of spread of the leaf-roll virus, using aphides as infecting agents, led to negative results, the aphides in these particular cases not doing well on the healthy plants, and no disease was contracted.

It has been noted that some lateral shoots, which the second generation of plants shows to have been diseased when removed from the original plant, fail to develop visible symptoms of disease while being grown as cuttings. As a rule, in fact, the original plant develops symptoms long before any of the infected cuttings do, and it is evident that the treatment the latter are subjected to delays the appearance of disease. Another point which stands out clearly from this work is that the appearance of symptoms on the young shoots of a newly infected plant has no connection either in time or space with the spread of the virus. Symptoms do not appear until about 4 weeks after grafting, and then only on a part of the plant, but the virus has already penetrated to all the portions of the stem in less than 14 to 15 days. Means are probably now available to show whether the virus travels faster to regions of active growth than to others.

Isolation of Streak and other Mosaic Diseases.

Atanasoff (3) has shown that streak is a very common disease in Holland, and that it is frequently present in certain varieties where it does little or no harm, and where its presence would never be suspected. This conclusion we can confirm, for we had already arrived at it independently. Thus the disease isolated from the variety Barley Bounty (which is a tolerant variety) in 1923, about the identity of which doubt existed (8), has been analysed to the extent that streak has been isolated from it, and there is a presumption that crinkle is present as well. Similarly streak has been proved to be present in plants of Irish Chieftain and Arran Chief, among other varieties, in which there was no apparent symptom of the disease.

Streak is a source of real anxiety in endeavouring to isolate other diseases, particularly mosaic, and we have made it a rule lately to isolate mosaic on plants of Arran Victory and Arran Comrade, on which the effects of streak, whether alone or combined with mosaic diseases, are very severe.

Another procedure, which is still in the experimental stage, relates to presumed combinations of streak and other mosaic diseases. It is based on the hypothesis that if streak is present a further infection with the same disease will not produce any alteration in symptoms; or perhaps if the primary symptoms are different, the secondary symptoms should be the same. This supposition, however, still requires proof.

Still another plan, which is now being adopted tentatively, is to graft certain control cores into healthy tubers of a variety like Arran Comrade, which is very susceptible to streak, and to grow a plant from the grafted Arran Comrade tuber as a control instead of from the core, or in addition to a plant from the core, if the latter has an eye. The object of this is to prove that the tuber originally grafted was free from streak, for if it belonged to a variety that tolerated this disease, the growing of a control plant from the core would not prove the disease to have been absent.

SUMMARY.

A reliable method is described of grafting a tuber by means of a core removed from another tuber and inserted into an opening of slightly smaller size in the former. Diseases of the mosaic group are transmitted by this means, and the regularity with which this happens is probably in direct proportion to the infectiousness of each disease. These diseases are sometimes separated from each other in the process. Leaf-roll is transmitted only rarely, and its entire suppression can apparently be brought about by incubating the grafted tubers at 20° C.

This method of grafting is well adapted to experiments which are designed to be completed within a single season, the tubers being planted in the open. Other applications are also discussed.

Other means of infecting tubers are mentioned, these being cleft-grafting carried out on the sprouts, which is useful in the case of leaf-roll; and the use of aphides, which have not been found sufficiently reliable for routine work, although three forms — *Myzus persicae, M. pseudosolani,* and *Macrosiphum solanifolii*—were proved to carry infection in this way.

For infecting stalks and foliage, cleft-grafts are reliable (except for streak sometimes) and transmit all diseases and combinations equally. Leaf-mutilation was found to transmit streak. Aphides were very unreliable agents of infection for leaf-roll, at least. The capsid, *Calocoris bipunctatus,* was again found to transmit leaf-roll.

A method is described whereby the rate of spread of a virus in the potato plant may be studied more exactly, by infecting single-stemmed plants at the top, and then removing the lateral shoots at intervals and growing them as cuttings; or, alternatively, by removing tubers at intervals. The leaf-roll virus was proved to have reached all parts of the stem and tubers from a scion grafted on at the top after an interval of more than 8 to 10 days and less than 14 to 15 days.

The occurrence of varieties tolerant to streak has been proved, and methods are suggested for securing other mosaics free from this disease, and for proving the presence of streak in tolerant varieties.

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DESCRIPTION OF PLATES.

Plate VIII.

Fig.

1. Method of core grafting, showing diseased tuber on left, healthy tuber containing a core without an eye from diseased tuber on right, and core with an eye from healthy tuber above.

2. A combination of leaf-roll and mosaic in the same plant, from which the mosaic was isolated by means of a core-graft, as shown in fig. 3. Approximate size of tuber planted is shown. Variety—President.

3. Plant infected with mosaic by means of a core from a tuber carrying leaf-roll and mosaic (see fig. 2). The leaf-roll was not carried over. Variety—President.

4 Non-inoculated healthy control plant grown from portion of the same tuber as produced plant shown in fig. 3.

5. A pair of single-stemmed healthy plants grafted at the top with scions from a diseased plant and ready for an experiment in which the lateral shoots are detached and grown as cuttings to determine rate of spread of virus.

Plate IX.

6 A healthy control plant produced by growing as a cutting the top of a plant cut off in the operation of grafting.

7. Experiment on rate of spread of leaf-roll virus in plant. Plant on left, which is healthy, is the produce of a tuber from a lateral shoot removed 10 days after grafting. Plant on right is diseased with leaf-roll, and is similarly produced from a shoot removed from same plant 15 days after grafting. Variety—British Queen.

8. Small plant on right is produced by a tuber from a plant infected with leaf-roll by means of capsids under conditions where aphides were excluded. Control plant, originally from half of same tuber, which is shown on left was grown under same conditions. Variety—Kerr's Pink.

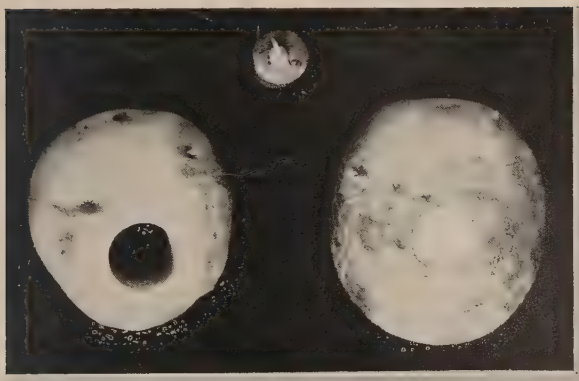

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 7.

FIG. 6.

FIG. 8.

No. 15.

AN EXAMINATION OF THE ERRORS INTRODUCED BY THE VARIOUS APPROXIMATE METHODS USED FOR ESTIMATING THE TOTAL QUANTITIES OF MILK AND BUTTER FAT PRODUCED DURING A LACTATION.

By E. J. SHEEHY, B.Sc., Hons. [Lond.], F.R.C.Sc.I., M.R.I.A.

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The literature dealing with the milk yield of the cow is varied and voluminous, and there are numerous references to the daily, monthly, and annual variations which appear in the yields of individual animals. Some of the variations can be accounted for in a number of ways, but the cause of others is not clear. The quantity of milk and butter fat yielded during the successive weeks of the milking period varies with the stage of lactation, the time of calving, the nature of the food, the state of the animal's health, and other circumstances. Superimposed on this continuous and gradual variation in yield is an extraordinary diurnal variation, the fundamental cause of which is connected with the changing activity of live protoplasm. Not only is there a double phase variation, but the digression from a mean yield, particularly in the case of daily variations, is irregular, and consequently short-cut methods of making correct determinations of the total yields of milk and butter fat over the entire lactation period are difficult, if not impossible, to devise. The only scientifically accurate method of arriving at the absolutely correct figures is by a determination of the individual daily yields for the lactation period, but such procedure involves an expenditure of time and labour which excludes this method from general application on the farm. Short-cut methods are essential, but they must be such as to give results to a fair degree of exactitude.

A number of suggestions have been made for the determination, with the minimum amount of labour, of the total milk or the total butter fat yield during the period of lactation. For instance, Wilson[1] proposes the multiplication of the figure representing the maximum yield of milk by a constant figure in order to obtain the complete lactation yields, and the writer has suggested[2] that a figure for fat percentage to represent a fair average for the whole lactation can be obtained by averaging the figures obtained during four or six successive days in the centre of the milking period. The application of Wilson's method of total yield determination has recently been discussed by Saunders.[3] Individual herd owners have adopted empirical methods of obtaining both the yields of milk and of butter fat by determining the weight of the milk and the percentage, and subsequently the weight of butter fat at intervals

[1] Principles of Stock-breeding, p. 110.
[2] Sc. Proc. R.D.S., vol. xv (N.S.), No. 40.
[3] Agric. Gazette and Modern Farming, December 21, 1923, p. 799.

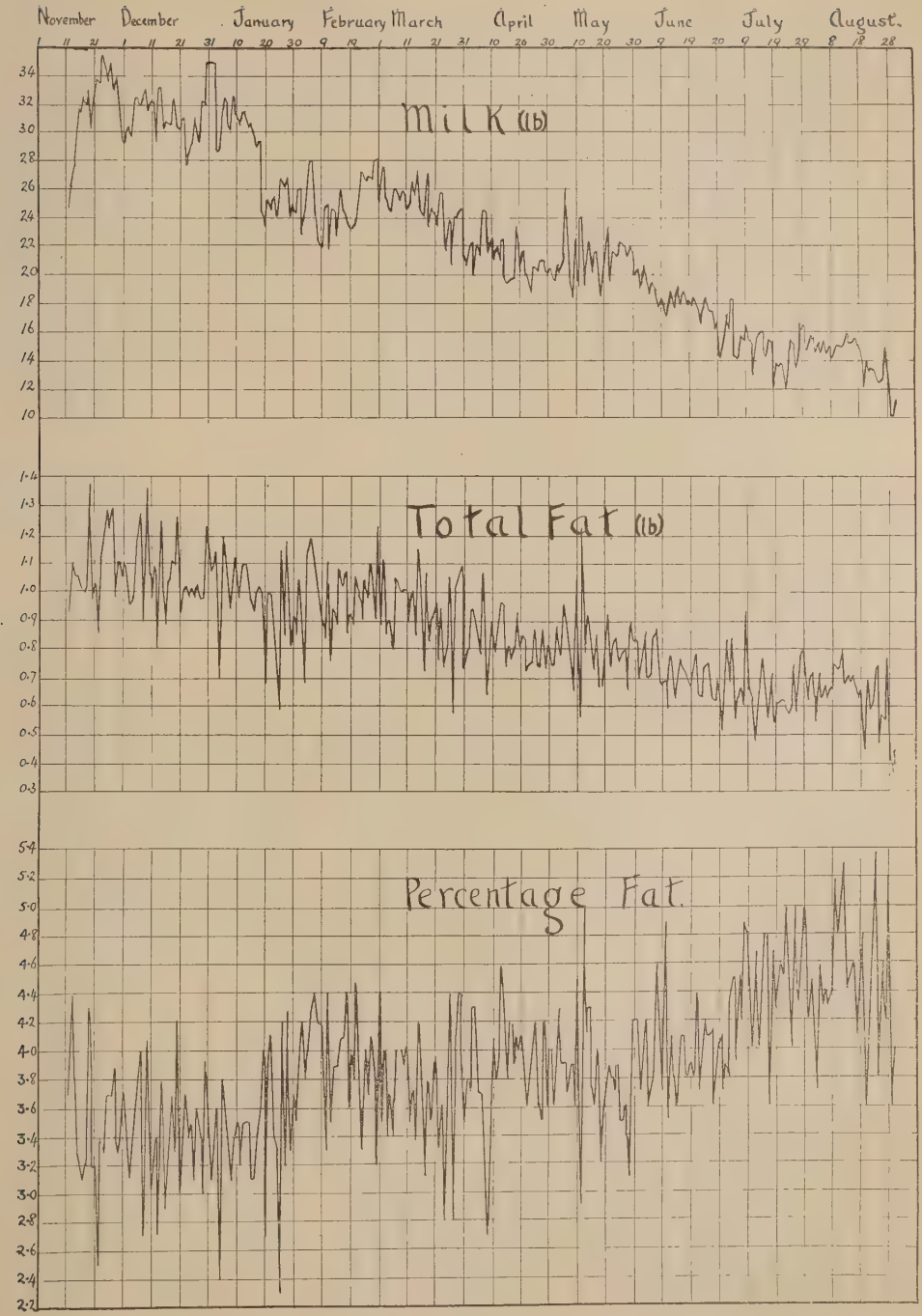
during the lactation, and Departments of Agriculture and milk testing federations in many countries apply a similar method in connection with the recording of milk and butter fat yields on a large scale among dairy farmers. The intervals of time elapsing between successive determinations of yield in the short-cut methods adopted by different organisations and individuals in a single country vary. The comparative accuracy of the empirical methods in operation and in those possible of suggestion is a matter of considerable interest, and the degree of accuracy to be expected in the results obtained by calculation from determinations made at various intervals of time is a matter of much practical importance.

The curves for yields of individual cows show quite a number of variations from those of the average of a herd, and it is difficult to find two cows in the case of which the curves of yield are parallel. A graphical representation of a more or less typical yielder—cow 17—is illustrated in the figure, in which is shown not alone the general trend of the curves during the lactation, but also the diurnal variations.

To investigate the comparative accuracy of the various methods of determining lactation yields a number of cows—cross-bred shorthorns, five Spring and three Autumn calvers—were selected from the dairy herd at the Ballyhaise Agricultural Station in 1921, and during the 1921–22 milking season the milk of each cow was weighed twice daily, i.e. at each milking, and the percentage of fat of a composite morning and evening daily sample was determined by the Gerber tester method. The milking was superintended by Mr. R. Collins, of the College staff, who weighed the milk and performed the fat tests, and whose part in this investigation I am glad to acknowledge. The cows were on grass in Summer and house fed in Winter, when they were also allowed on to pasture for some time each day. The daily yields of milk and butter fat were recorded for each cow throughout the entire lactation period. Tables were made and graphs drawn to represent the daily yield of milk, the total daily yield of fat, and the percentage of fat in the daily milk of each cow; and from these tables and graphs the calculations presented in the following pages were made. From the daily record the total yields of milk and butter fat for the entire lactation period were calculated in the case of each cow, and from these two quantities the true percentage of fat was determined.

Similar determinations were made in the case of a number of cows from the herd of the Albert Agricultural College, Glasnevin, figures for which had previously been collected by the writer.

Using the tables and graphs of daily yield the figures for certain dates selected at intervals of time were extracted, and from these figures the total lactation yields were calculated. The results thus obtained were identical with those which would have been obtained had the milk and butter fat yields been actually determined on these particular selected dates only. Using the tables it was possible, so to speak, to visit the herds at selected intervals of time and note the milk and butter fat yields; and by altering the intervals and the system of calculating the final results, the determination of the figures for the complete yields, according to every conceivable short-cut method, was rendered possible. Comparison of these results with the true yields, as calculated from the successive daily figures, gave the degree of accuracy to be expected from each short-cut method.


November December January February March April May June July August.
1 11 21 1 11 21 31 10 20 30 9 19 1 11 21 31 10 20 30 10 20 30 9 19 29 9 19 29 8 18 28
34 32 30 28 26 24 22 20 18 16 14 12 10
Milk (lb)
1·4 1·3 1·2 1·1 1·0 0·9 0·8 0·7 0·6 0·5 0·4 0·3
Total Fat (lb)
5·4 5·2 5·0 4·8 4·6 4·4 4·2 4·0 3·8 3·6 3·4 3·2 3·0 2·8 2·6 2·4 2·2
Percentage Fat.

Experimental Results.

The true yields of milk and butter fat, the true percentage of fat for the lactation period, and other data relative to the Ballyhaise Station cows utilised in this investigation are given in Table I.

Table I.

No. of cow.	Date of calving.	No. of weeks in milk.	True yields for the Lactation period. Milk. (lbs.)	Butter fat. (lbs.)	True percentage of fat for the Lactation period.
23	14 Sept.	45	9308	312·3	3·4
17	10 Nov.	42	6656	250·5	3·8
41	1 Dec.	45	9344	390·6	4·2
21	21 April	40	7073	270·6	3·8
32	22 Mar.	44	7888	286·2	3·6
60	29 „	39	5061	216·5	4·3
72	7 April	44	5406	192·7	3·6

The true total milk yield was obtained by totalling the figures representing the daily yields for the lactation.

The daily yield of butter fat was obtained from the daily weight of milk and the percentage of fat contained therein, the true lactation yield being the sum of the individual daily yields.

The true percentage of fat was calculated from the true total yields of milk and butter fat for the entire lactation period.

Milk.—In elaborating short-cut methods of arriving at total yields it is convenient to consider the figures for milk and butter fat separately. Having determined the true total yield of milk for the lactation period the results obtainable from single daily weighings each week were then calculated. The weight of milk yielded on Wednesdays, for example, multiplied by the figure 7, gave a certain result for total yield, but, since the successive daily yield varies, a different result is obtainable from the samples taken on Thursdays, and so on for the other days of the week. It was necessary, therefore, to obtain seven results for each cow by the method of weekly weighings. The variation of these results on the true total milk yield for the lactation period was calculated and represented as a percentage error, a figure which indicates the degree of accuracy to be expected in the yield of milk obtained by calculation from weekly recording.

Table II gives the percentage error in the yields obtained for each cow from each of the seven sets of weekly determinations.

Table II.

Variation on True Yield or Percentage Error.

	Cow 23.	Cow 17.	Cow 41.	Cow 21.	Cow 32.	Cow 60.	Cow 72.
Weighing the milk on Sundays ...	+0·5	−0·3	+0·7	−0·8	−0·2	−0·3	−2·1
„ „ „ „ Mondays ...	+0·2	+1·0	+0·5	−0·4	−0·4	−0·4	−1·0
„ „ „ „ Tuesdays ...	−1·6	−0·4	−0·8	+0·3	0·0	−0·1	−0·1
„ „ „ „ Wednesdays	+1·2	+0·1	+0·5	+1·5	+1·3	+1·5	+0·5
„ „ „ „ Thursdays ...	+0·1	−0·8	−0·1	−0·8	+0·2	−0·3	−0·4
„ „ „ „ Fridays ...	−0·3	+0·2	+0·8	+1·4	+0·9	+0·2	−0·2
„ „ „ „ Saturdays ...	0·0	0·0	−1·6	−1·0	−1·5	−0·6	+1·2

The total lactation yields calculated from weighings made on one day at intervals of two weeks were next determined, and again seven results were obtained for each cow according to the day of the week the milk yield was weighed.

The lactation yield of milk in this case was obtained by multiplying the totals of the fortnightly weighings by a fraction representing the following expression:—total number of weeks in milk divided by the number of weighings made and multiplied by 7.

Determinations of the total lactation yield of milk from weighings made on one day at intervals of three, four, five, and six weeks respectively were then made, and the percentage variation on the true yields was determined. The percentage error which occurred in the results obtained when the intervals were exactly six weeks is shown in Table III, while the maximum and the average errors introduced by using the various methods will be found in Table IX.

TABLE III.

VARIATION ON TRUE YIELD OR PERCENTAGE ERROR.

	Cow 23.	Cow 17.	Cow 41.	Cow 21.	Cow 32.	Cow 60.	Cow 72.
Weighing the milk on Sundays ...	+2·2	+4·1	−4·6	−1·7	+4·8	−4·1	−8·2
„ „ „ „ Mondays ...	−1·9	−4·7	−6·7	−5·1	+5·4	−1·1	+2·5
„ „ „ „ Tuesdays ...	−0·3	+2·2	+6·2	+6·8	−1·8	+1·7	−1·4
„ „ „ „ Wednesdays	+2·5	+1·6	−1·9	−2·0	+3·0	−4·2	−1·1
„ „ „ „ Thursdays	−1·1	−2·4	−2·4	−8·2	−3·0	−4·2	+1·3
„ „ „ „ Fridays ...	0·0	+1·1	+4·3	+0·4	+1·5	+3·1	+1·7
„ „ „ „ Saturdays	−1·1	+2·4	−5·0	−3·5	−6·8	−3·7	0·0

Butter Fat.—The lactation yields of total fat, obtained according to a number of short-cut methods, were investigated for degree of accuracy in a similar manner to that which was used in the case of total milk. From the tables of the continuous daily yields of fat the figures for certain dates at stated intervals of time were abstracted, and, as before, a number of determinations were made for each cow by each individual method employed according to the day or date on which the sampling started. The exact procedure in making the calculation for total butter fat yield for the lactation was as follows:—

(1) When tests for fat were made at intervals of one week the total fat produced on each day of test was determined from the milk yield and percentage of fat: the fat totals thus ascertained were added and the sum multiplied by 7.

(2) When tests for fat were made at intervals of two weeks the weights of fat produced on the dates of test were determined and totalled: the grand total was then multiplied by the following expression:—number of weeks in milk divided by the number of tests made and multiplied by 7.

(3) When tests for fat were made daily at intervals greater than two weeks the weights of fat produced on the dates of test were determined and totalled: the total milk yield of the same dates was determined; and by a simple proportion calculation, using these two totals and the true lactation milk yield (as determined from daily weighings) a figure for lactation fat yield was obtained. It would have been more in keeping with practical farm procedure to utilise the figure for lactation milk yield obtained from weekly weighings, but seven sets of weekly weighings were available, and calculations would have to be made for each and all of them. The true lactation milk figure was utilised in preference, and an error, which was thus intentionally withdrawn

for convenience, was afterwards added by superimposing on the error associated with the determined lactation fat the error incidental to the weekly method of ascertaining lactation milk yield.

On account of the erratic variation of milk fat on successive days a method similar to (1) or (2) above, if applied where the intervals are long, would be liable to large errors. Consequently method (3) was chosen for greater accuracy when the intervals exceed a fortnight.

Seven results for lactation fat were obtained for each cow from weekly tests, and fourteen from fortnightly tests.

The percentage of error in the results obtained from tests made at intervals of one week is represented in Table IV.

TABLE IV.

VARIATION ON TRUE YIELD OR PERCENTAGE ERROR.

	Cow 23.	Cow 17.	Cow 41.	Cow 21.	Cow 32.	Cow 60.	Cow 72.
Testing on Sundays	+0·8	−0·3	−2·2	−0·3	−0·2	+1·5	+0·7
„ „ Mondays	−3·3	−0·4	+2·2	+1·0	+0·3	+0·7	−2·8
„ „ Tuesdays	+2·5	−0·7	−0·2	−1·0	+0·9	−0·2	+2·4
„ „ Wednesdays ...	−2·5	+1·4	+2·2	+3·8	+0·3	−0·2	−0·3
„ „ Thursdays ...	+2·8	−2·2	0·0	−2·9	−1·7	−1·8	−3·1
„ „ Fridays	−1·8	+1·0	−0·8	+2·1	+1·8	+0·2	+2·6
„ „ Saturdays ...	+1·0	+0·8	−2·2	−2·5	−1·2	−0·3	+0·4

The results obtained from tests made at intervals of three, four, five, and six weeks respectively were based on a calculation which, as outlined on page 189, presupposes the knowledge of the true figure for lactation milk yield. Table V shows the percentage error where the interval between the tests was six weeks. A more useful result is obtained by adopting the lactation milk figure ascertained from weekly weighings. Consequently, in considering the percentage of error incidental to the various short-cut methods of determining lactation fat, the error in the lactation milk, as shown in Table II, was added to those errors which appear in the results as determined by method (3), page 189, where the interval between the tests exceeds two weeks. Such procedure was adopted in the compilation of the figures in Table X. Thus, if the maximum and average variation in the results obtained by a particular method, as, for example, in the results shown in Table V, be represented as follows:—maximum $+a$ and $-a_1$, average $\pm c$, and the corresponding items in Table II be as follows:—maximum $+x$ and $-x_1$, average $\pm z$, then the maximum and average errors for the purpose of Table X become

maximum $+(a+x)$ and $-(a_1+x_1)$;

average $\pm\sqrt{c^2+z^2}$.

TABLE V.

VARIATION ON TRUE YIELD OR PERCENTAGE ERROR.

	Cow 23.	Cow 17.	Cow 41.	Cow 21.	Cow 32.	Cow 60.	Cow 72.
Testing on Sundays	+8·4	+6·7	+2·0	−4·1	−0·9	−0·2	+3·5
„ „ Mondays	+3·2	−3·6	−1·8	+5·0	−0·2	−0·7	−10·0
„ „ Tuesdays	+0·3	+1·0	+7·3	+4·8	−3·2	−0·9	+0·2
„ „ Wednesdays ...	+2·5	−2·4	−1·2	+0·4	+4·3	+1·6	+6·6
„ „ Thursdays ...	−5·7	+0·8	+0·2	−3·2	+0·8	−1·0	+5·6
„ „ Fridays	−0·8	−0·1	+2·7	−3·6	−4·6	+3·4	+4·7
„ „ Saturdays ...	+0·3	−2·1	+3·8	+10·1	+8·5	−3·9	−1·9
„ „ Sundays, starting 1 week later	−10·0	+2·1	−0·2	−1·8	−0·8	+1·2	−0·3
„ „ Mondays „ „	−5·7	−6·0	+1·3	−5·8	−8·7	+2·1	−2·0
„ „ Tuesdays „ „	+2·5	+0·4	−3·2	−1·6	−2·2	+2·5	−2·3
„ „ Wednesdays „ „	−9·2	−0·[illegible]	−0·2	+4·7	+2·7	−1·7	−3·7
„ „ Thursdays „ „	+2·7	−1·4	+3·3	−0·6	+2·5	−4·1	−3·6

Table VI gives the percentage of error in the results obtained from five tests made at approximately regular intervals during the lactation, and Table VII gives the error in results from four tests made at irregular intervals.

TABLE VI.

VARIATION ON TRUE YIELD OR PERCENTAGE ERROR.

	Cow 23.	Cow 17.	Cow 41.	Cow 21.	Cow 32.	Cow 60.	Cow 72.
1st series of tests	+0·8	+8·0	−0·4	−3·5	+9·3	−0·7	−5·8
2nd „ „ „	−6·0	−1·5	−0·8	−9·5	−1·0	−3·0	−2·5
3rd „ „ „	+2·0	−0·7	+1·2	−8·0	+7·1	+1·2	+6·7
4th „ „ „	+2·4	−3·5	+5·5	−2·5	−0·2	+0·5	−9·7
5th „ „ „	+3·9	+3·6	−4·7	+0·8	+2·2	−0·9	−3·1
6th „ „ „	+1·3	+0·4	−0·5	+0·7	−4·3	−3·3	−3·5
7th „ „ „	+5·4	+9·9	+1·1	+3·2	−6·4	+5·6	−3·3
8th „ „ „	−8·8	−8·4	+7·8	−1·9	+7·2	+0·5	+3·5
9th „ „ „	−5·3	+9·6	+9·4	−2·8	−1·0	−0·6	−9·9
10th „ „ „	−12·1	+4·0	+4·9	−1·3	−1·7	+1·4	−8·5
11th „ „ „	+10·7	−2·1	−2·0	+2·2	+0·3	−0·7	−2·4
12th „ „ „	−0·2	+1·9	−1·0	+11·5	−2·9	+0·2	−5·2

TABLE VII.

VARIATION ON TRUE YIELD OR PERCENTAGE ERROR.

	Cow 23.	Cow 17.	Cow 41.	Cow 21.	Cow 32.	Cow 60.	Cow 72.
1st series of tests	−1·7	0·0	−1·8	−10·6	+2·1	−4·4	−8·7
2nd „ „ „	+1·2	+2·0	+4·3	+11·0	+4·1	−5·8	−0·1
3rd „ „ „	−2·7	+0·9	+3·9	−1·2	−1·3	+0·6	+2·9
4th „ „ „	+2·9	+4·9	−0·6	+13·2	+0·2	+1·5	−7·9
5th „ „ „	−7·8	−1·7	−7·9	−15·9	−14·1	−7·4	+11·3
6th „ „ „	−5·9	−0·4	−1·1	−7·7	−5·1	+2·3	−3·2

The idea suggested itself that, in view of the peculiar daily variations in yield a more accurate figure for total lactation fat would be obtained were the tests made on two successive days. Determinations were made from two successive day tests made at intervals of six weeks and also at intervals of seven weeks, and the errors in the results from the two-day method at six weekly intervals are shown in Table VIII.

Table VIII.

Variation on True Yield or Percentage Error.

	Cow 23.	Cow 17.	Cow 41.	Cow 21.	Cow 32.	Cow 72.
Testing on Sundays and Mondays ...	+3·1	−5·1	0·0	−7·1	−1·6	−0·7
„ „ Mondays and Tuesdays ...	−0·3	−0·6	+3·9	+7·9	−3·4	+3·4
„ „ Tuesdays and Wednesdays ...	+3·3	+0·9	+4·6	−7·7	+0·2	+2·2
„ „ Wednesdays and Thursdays ...	+6·4	+4·1	+3·5	−2·6	+0·2	+3·0
„ „ Thursdays and Fridays ...	+2·7	+3·6	−0·5	+1·9	+0·4	−1·7
„ „ Fridays and Saturdays ...	−0·1	+1·5	+1·9	−0·7	−0·8	−5·0
„ „ Saturdays and Sundays ...	−0·1	+2·7	+4·2	−5·7	0·0	+3·2
„ „ Sundays and Mondays—starting 1 week later	+4·0	−0·4	−0·8	−6·9	+0·7	+3·9
„ „ Mondays and Tuesdays „	−7·3	−0·1	+1·7	+0·9	−1·0	+2·9
„ „ Tuesdays and Wednesdays „	+4·7	−1·7	+4·8	+1·1	−0·8	+1·4
„ „ Wednesdays and Thursdays „	−6·3	+1·9	+5·5	−7·4	+1·3	−0·1
„ „ Thursdays and Fridays „	−7·1	−3·6	+4·0	−7·5	−3·8	−1·1
„ „ Fridays and Saturdays „	+5·0	−3·6	−0·2	−0·8	−4·6	−2·2
„ „ Saturdays and Sundays „	−5·7	−0·5	−0·9	+2·0	+1·2	−2·0

Determinations of the figures necessary for the purpose of comparing the accuracy of the different short-cut methods of arriving at the lactation milk and butter fat, and tabulations similar to the foregoing were made for five cows from the herd at the Albert Agricultural College, Glasnevin, and the discussion of results which follows is based on the results obtained from both the Glasnevin and the Ballyhaise herds.

Discussion of Results.

The comparative accuracy of the various methods employed in the determination of the total yields of milk and butter fat for the lactation period is revealed in the figures representing the variation of the calculated on the true yield, i.e. in the percentage error. Six short-cut methods of obtaining lactation milk yield and ten short-cut methods of arriving at the total lactation fat yield were applied.

Milk.—In the case of milk, weighings were made, so to speak, on one day each week, and on one day each two, three, four, five, and six weeks respectively, and determinations of lactation milk were made therefrom. When the errors resulting in the case of all cows tested according to any one short-cut method are collectively considered the maximum and the average error associated with that method, at least as far as the limited results available indicate, may be stated. The comparative extent of the variation which occurs in the employment of the several short-cut methods is shown in Table IX.

TABLE IX.

ERROR IN ASCERTAINED LACTATION MILK YIELD.

Method employed.	Maximum error (per cent.).	Average error (per cent.).
Weighing one day's yield—		
At intervals of one week ...	+1·5 and −2·1	± 0·64
„ „ „ two weeks ...	+3·0 „ −4·3	± 1·44
„ „ „ three weeks ...	+4·3 „ −5·0	± 1·48
„ „ „ four weeks ...	+5·4 „ −5·8	± 2·37
„ „ „ five weeks ...	+8·0 „ −6·3	± 2·47
„ „ „ six weeks ...	+6·0 „ −8·2	± 3·08

It is seen that results, which approximate very closely to the true figures, are obtained where weekly weighings are adopted, and that the error occurring in the results increases as the length of the interval between the weighings is made greater. In a somewhat similar investigation[1] made in France the greatest variation obtained for individual cows was as follows:—

Weekly tests	3·5 per cent.
Fortnightly tests	5·8 „
Three-weekly tests	8·1 „
Monthly tests	9·9 „

Butter Fat.—In the case of milk fat, daily tests were made, so to speak, at intervals of one, two, three, four, five, and six weeks respectively, and the total lactation fat in each case determined therefrom. In addition, the following methods were also investigated:—testing five times in the lactation at fairly regular intervals, testing four times in the lactation at irregular intervals, and testing on two consecutive days at intervals of six and seven weeks respectively. Taking the recorded cows collectively the maximum and average errors which occur in the ascertained lactation fat yields are given in Table X.

TABLE X.

ERROR IN ASCERTAINED LACTATION FAT YIELD.

Method employed.	Maximum error (per cent.).	Average error (per cent.).
Testing one day's yield—		
At intervals of one week ...	+ 3·8 and − 3·3	± 1·40
„ „ „ two weeks ...	+ 5·6 „ − 4·9	± 2·60
„ „ „ three weeks ...	+ 9·4 „ − 9·8	± 2·88
„ „ „ four weeks ...	+10·6 „ −11·2	± 2·98
„ „ „ five weeks ...	+10·7 „ − 9·4	± 2·88
„ „ „ six weeks ...	+11·6 „ −12·1	± 3·16
Five times during lactation at nearly regular intervals ...	+12·1 „ −14·2	± 3·85
Four times during lactation at irregular intervals	+14·7 „ −18·1	± 4·64
Testing two consecutive days' yield—		
At intervals of six weeks ...	+ 9·2 „ − 9·6	± 2·88
„ „ „ seven weeks ...	+ 9·0 „ − 9·6	± 2·88

[1] Revue de Zootechnie, la revue des eleveurs, year 3, No. 10, pp. 239–247. Paris, 1924. (From the Int. Rev. Sc. and Pr. Agric., New Series, vol. iii, No. 2, p. 554.)

On comparing the accuracy of the different short-cut or approximate methods of determining the lactation butter fat it is seen that, as far as the cows under investigation are concerned, weekly tests give a result which approximates closely to the true figure. The results from fortnightly tests diverge further, though not considerably more than the weekly test results from the true yield, while those calculated from tests taken at intervals greater than a fortnight are liable to give single results which are seriously above or below the correct figure. As the interval between the tests is widened the average error of the single results increases in intensity at a slower rate than does the maximum error. Testing on two consecutive days at long intervals gives no better result than the method of taking the same total number of tests collected at shorter intervals from single rather than from two consecutive day samples. The results obtained by the method of taking a small number of samples at random during the lactation may, in single instances, digress to an alarming extent from the correct figure though the average error, even according to this method, remains a comparatively small figure.

In the French investigations previously referred to the maximum variations in fat content observed were as follows:—

Test made weekly	4·3	per cent.
„ „ fortnightly	6·6	„
„ „ three-weekly	11·7	„
„ „ monthly	9·9	„

Where questions of practicability demand long intervals between the fat tests it is advisable to record the milk yield weekly, because in the calculation for total lactation fat the recorded milk yield figure is utilised, and it is important that the smallest possible error be thus introduced into the calculation. The extent of the error allowable in lactation milk and butter fat yields depends upon the purpose for which the results are sought, which consequently must determine the method to be employed in making such determinations. In finding out the value of the dairy cow for milk and butter production it is essential to collect the figures for both milk and butter fat for each individual animal over each lactation, and, since any useful short-cut scheme of determining yields must be applicable to both milk and milk fat, the method of weighing the milk and testing samples for fat at intervals appears to be the only one capable of fulfilling the double purpose with a reliably fair degree of exactitude.

No. 16.

PRELIMINARY NOTE ON THE PHYSIOLOGICAL ASPECTS OF CERTAIN UNDESCRIBED STRUCTURES IN THE PHLOEM OF THE GREATER YAM, *DIOSCOREA ALATA*, LINN.

By T. G. MASON, M.A., Sc.D.

(Plates X and XI.)

[Read December 15, 1925. Printed January 20, 1926.]

In connexion with observations recently made on the rate of sugar transport in the Greater Yam, it was observed that the phloem was of a markedly abnormal type. As no reference to some of the structures found appears in the literature, a short account of them may be of interest. Though their investigation is in many respects incomplete, yet as a considerable period must elapse before the writer can hope to complete it, and as the structures to be described may help in the elucidation of the function of the phloem, it has been decided to publish a note at the present stage of the enquiry.

When a longitudinal section is made through a node (fig. 1) it is seen that there are two masses of interlacing vessels and xylem parenchyma, each forming a plexus close to the insertion of the petioles. It will be convenient to refer to them as the *wood-plexus* (S). Beneath them are a number of structures which are often ovoid, but may be very irregular in shape. They will be referred to as the *bast-glomeruli* (B). They are usually very conspicuous beneath each of the wood-plexus, but also occur above them. On the right of fig. 1 it will be noticed that the bast-glomeruli are situated between the sieve-tubes that approach from each side of the node.

Their relation to the sieve-tubes is illustrated in figs. 2 and 4. In the former a number of giant sieve-tubes (A) are shown, each of them terminating in a glomerulus (B). The sieve-plates of one of these giant sieve-tubes may be seen in fig. 3. The sieve-tubes on the other side of the glomeruli do not appear in fig. 2, but in fig. 4 the sieve-tubes (A) on each side of the glomeruli (B) are in the same plane, and it can be seen that they are in communication with the sieve-tubes on each side through a large number of approximately parallel deeply-stained cells (D).

The nature of the cells connecting sieve-tube and glomerulus is indicated in fig. 5. In contact with the sieve-tube (A) is a cell (E) shaped like a funnel with a twist in the stem; the latter being attached to the sieve-plate. Three nuclei can be observed in this cell, which may be termed the *funnel-cell*. On the lip of the funnel are a great number of short stout cells (D) that stain deeply with haematoxylin. Frequently these cells, for which the term *bast-tubules* is proposed, are of considerable length (cf. fig. 6). They are, moreover, often directly attached to the sieve-plate, the funnel cell being absent. In contact with the tubules (figs. 5 and 7) are the long prismatic cells that constitute the glomerulus. They present the appearance of being constricted or twisted along a definite line which forms the surface of the glomerulus, within

which their cell walls are thin and stain less deeply than the portion lying outside. It will be noticed (fig. 7) that some of the cells of the glomerulus are binucleate. A transverse section through a glomerulus is shown in fig. 8. The thin walls and conspicuous deeply-stained nuclei of the cells of the glomerulus suggest that it is a region of metabolic activity.

A number of species of the genus *Dioscorea* have been examined in order to ascertain whether glomeruli are distributed throughout the genus or not. The species examined are as follows:—

Stenophora.
- *D. villosa,* Linn.
- *D. Tokora,* Makino.
- *D. nipponica,* Makino.

Opsophyton.
- *D. bulbifera,* Linn.
- *D. sativa,* Linn.
- *D. macroura,* Harms.

Lasiogyne.
- *D. discolor,* Herb. Berol. ex Kunth.

Lasiophyton.
- *D. dumetorum,* Pax.

Enantiophyllum.
- *D. opposita,* Thunberg.
- *D. decaisneana,* Carrière.
- *D. japonica,* Thunberg.
- *D. alata,* Linn.

It was found that the structure of the glomerulus, where present, is essentially similar to that already described for *D. alata,* but that in *D. dumetorum* the structure is rather more complex. The cells that constitute the glomerulus in this species (fig. 9) tend to be thrown into a series of folds so that a number of parallel lines appear to cross it. Glomeruli were not found in *D. villosa* and *D. Tokora,* both of which belong to the section Stenophora. As *D. nipponica* is also a member of this section, the presence of glomeruli in this species requires confirmation, as some doubt exists concerning the identity of the material examined. Glomeruli were found to be present in every section except the Stenophora. Now in the two species in which glomeruli were not observed, and also, it may be added, in *Tamus communis,* where they are apparently absent, the wood-plexus are relatively inconspicuous in comparison with those in the species where glomeruli are present. It would seem that the development of the glomeruli is correlated with that of the wood-plexus. It may be that their function [and the thin walls of the cells of the glomerulus and their deeply-stained nuclei are in accord with the view] is secretory. Possibly enzymes are secreted which are transmitted *via* the xylem parenchyma to the interlacing tracheae of the wood-plexus. This is, of course, mere speculation. It is equally probable that glomeruli function as organs abstracting coagula which are transmitted to and stored in the sieve-tubes. It seems probable, whatever their function may be, that it is in some way correlated with that of the wood-plexus (4). Evidence seems to accumulate that the normal translocation of solutes is only possible where both xylem and phloem are present. It will be evident that the presence of glomeruli forming a barrier at every node between the sieve-tubes must present an insuperable obstacle to the rapid movement of carbohydrates longitudinally in the phloem. It will be also evident

that the interlacing masses of tracheae which constitute the wood-plexus do not favour the suggestion (2) that tension in the transpiration stream is responsible for the transport of carbohydrates.[1]

Both Kny (3) and Bucherer (1) have shown that the phloem in the bundles of certain members of the Dioscoreaceae is subdivided into a number of strands. Figs. 10 and 11 will show how the phloem is arranged within the bundles of *Dioscorea alata*. There is great variability not only in this respect but also in the stelar pattern of the various species of the genus *Dioscorea* examined in the course of the present work. It will be seen (figs. 10 and 11) that each phloem strand is encircled by a thick sheath of sclerenchyma—a circumstance which must entail a considerable measure of physiological isolation of each of these strands. The presence of such a sheath does not suggest any functional correlation between xylem and phloem in the internodes. As this sheath is absent from, or poorly developed in, the phloem strands of certain species which possess glomeruli, there is apparently no connexion between the sclerenchyma and glomeruli. Gaps do, however, occur in the encircling sclerenchyma in *D. alata*, and commissures of tubular cells have been observed connecting not only contiguous strands, but even those in neighbouring bundles. Moreover, reference to fig. 12 will show how a sieve-tube may directly impinge on a vessel. It is of interest to note that the phloem in the main veins of the leaf and the petiole is subdivided into three strands. Reference may be made to the difficulty experienced by the writer in distinguishing between vessels and sieve-tubes. Inspection of figs. 2 and 12 will show how similar, both in transverse and longitudinal section, are the two. It was not until it had been ascertained that dyes are not transmitted through the sieve-tubes, and their connexion with typical sieve-tubes in the leaf and the presence of undoubted sieve-plates had been demonstrated, that the presence of phloem could be admitted.

In conclusion, I have pleasure in acknowledging my indebtedness to Professor H. H. Dixon for his helpful criticism and for placing the facilities of his laboratory at my disposal. I am also indebted to Dr. Hill, Director of the Royal Botanic Gardens, Kew, for providing a great deal of the material used in the work, and to Mr. J. H. Burkill for information and advice.

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[1] It is, of course, possible that differences in tension in neighbouring tracheae are not eliminated by the wood-plexus.

DESCRIPTION OF PLATES.

[All microphotos.]

Plate X.

Fig.

1. L. S. through node showing wood-plexus (S), bast-glomeruli (B), sieve-tubes (A), and vessel (C). × 6.
2. L. S. at node showing giant sieve-tubes (A), bast-glomeruli (B), and vessels (C). × 27.
3. Oblique compound sieve-plate. × 400.
4. L. S. at node of young stem showing sieve-tubes (A), bast-tubules (D), and glomeruli (B). × 90.
5. L. S. at node showing sieve-tube (A), funnel-cell (E), bast-tubules (D), and cells of glomerulus (B). × 310.
6. T. S. at node showing sieve-tubes (A), bast-tubules (B), and glomeruli (D). × 72.

Plate XI.

7. Showing bast-tubules (A) and cells of glomerulus (B). × 470.
8. T. S. through glomerulus. × 370.
9. Compound glomerulus of *D. dumetorum*. × 63.
10. T. S. through portion of internode showing arrangement of bundles; vessels (C), phloem strands (A), and cortex (F). Sclerenchymatous sheath is shown as a black ring encircling the phloem strands. × 16.
11. T. S. through bundle showing arrangement of phloem strands (A). × 40.
12. T. S. through part of bundle showing phloem strand (D), abutting on vessel (C), and sclerenchyma sheath (F). × 135.

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No. 17.

THE ESTIMATION OF ORGANIC MATTER IN WATER BY MEANS OF POTASSIUM BICHROMATE AND SULPHURIC ACID.

By W. E. ADENEY, D.Sc., F.R.C.Sc.I., F.I.C.,

AND

MISS B. B. DAWSON, A.R.C.Sc.I.

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With the object of devising a simple method for estimating the total amount of organic matter in a clean or polluted water, the authors have carried out a series of experiments to ascertain if it be possible to effect its complete, or nearly complete, oxidation to the end products, carbon dioxide, water, and ammonia by treatment with potassium bichromate and sulphuric acid, and to estimate its amount in terms of the quantity of oxygen absorbed by it from the bichromate.

A number of experiments have been carried out with tap and river waters, including samples of tidal waters, with sewage liquors and effluents, and with simple solutions in tap water of known organic substances, and it has been found that the reaction between the bichromate and organic matter can be completed by digestion of the latter with an excess of bichromate in the presence of a sufficient concentration of sulphuric acid at a temperature of about 100° to 110° C. on an iron plate for about two hours, or on a water-bath for two to three hours.

It has also been found that when duplicate determinations of the oxygen absorbed by the organic matter in a given sample were made, closely concordant results were obtainable.

Test experiments with simple solutions of organic substances of known strength in tap water have been made in the following manner:—

50 c.c. of the solution were treated with a quantity of sulphuric acid, just sufficient to decompose the chlorides present, and evaporated in a porcelain dish down to about 5 c.c., on a water-bath, to expel all but small traces of the hydrochloric acid that is liberated from the chloride by the sulphuric acid, and to reduce the volume of the sample to a small bulk.

After the evaporation has been completed, the dish and its contents are cooled, and a mixture of 50 c.c. N/4 potassium bichromate solution and 50 c.c. sulphuric acid (concentrated acid previously diluted with an equal volume of distilled water) is added. The dish is placed on an iron plate, and digested at temperatures from 100° C. to 110° C. for about two hours, the liquid being well stirred from time to time, and the usual precautions taken to prevent contamination by dust. After digestion for about two hours, when the reaction between the organic matter and the bichromate will usually be found to have been completed, even in the case of the suspended organic matter in sewage liquor, the dish is removed, and its contents cooled and diluted

with water. The excess of bichromate is then titrated by means of ferrous sulphate.

It is necessary to make blank experiments with each set of analyses, and to make corrections for them. The results of two such tests are shown at the foot of the table. The other figures give the corrected values.

The quantities of oxygen absorbed by a number of simple solutions, when treated as above described, are given in the appended table, expressed in parts by weight per 100,000 parts of the solution :—

Solution.	Experiment.	Oxygen absorbed from acid bichromate per 100,000 parts.	
		Found.	Calculated.*
I. Rochelle salt	1	27·15	28·4
	2	27·2	
II. Asparagin	1	57·8	64·0
	2	57·6	
	3	58·3	
	4	58·1	
	5	58·6	
	6	58·5	
	7	58·5	
III. Sulphanilic acid ...	1	127·5	129·5
	2	127·2	
	3	127·3	
	4	127·3	
IV. Resorcinol	1	172·1	181·8
	2	172·5	
	3	172·2	
	4	171·3	
	5	171·3	
	6	172·6	
	7	172·0	
V. Gelatin	1	132·6	
	2	132·2	
	3	132·4	
	4	132·4	
	5	132·5	
	6	132·0	
VI. White of egg	1	144·4	
	2	143·8	
	3	144·2	
	4	144·3	
	5	143·9	
	6	144·2	
Tap-water, blank ...	1	6·0	
	2	6·9	

* Calculated on the assumption that the end products of oxidation of the organic substances were carbon dioxide, water, and ammonia.

Each of the solutions I to V contained 1 grm. of substance per litre. Solution VI contained 10 grms. of white of egg per litre.

Tap-water was employed in the preparation of each solution.

The above results show that for the substances examined, having regard to the multiplication of experimental errors, the oxidation in each case, under favourable conditions of experiment, was nearly complete to the end products, carbon dioxide, water, and ammonia; and that the quantity of oxygen absorbed by like volumes of each solution is sufficiently constant for practical purposes in water analysis.

One of the authors has frequently employed the method for the examination of polluted waters, and has been led to the belief, from the results that he has obtained, that the organic matter in normal sewage liquors is likewise nearly completely oxidised to the like three end products when treated in like manner.

Good results are also obtained when the digestion of the organic matter with the acid bichromate is carried out on a water-bath. It is then advisable to commence the digestion with a higher concentration of acid—2 volumes of concentrated sulphuric acid diluted with 1 volume of water. The reaction is usually completed after about three hours' digestion in the case of sewage liquors containing organic matter in suspension.

The following results were obtained after digestion for about three hours on a water-bath :—

Solution.	Experiment.	Oxygen absorbed from acid bichromate per 100,000 parts.
1*a*. Rochelle salt ...	1	26·5
	2	26·2
VII. White of egg ...	1	125·8
	2	133·2
VIII. Sewage liquor ...	1	28·0
	2	28·0
	3	25·6
	4	26·4
	5	28·0

Solution VII contained 10 c.c. of white of egg per litre.

The sewage liquor was collected from a sedimentation tank.

It must be borne in mind, when considering the foregoing results, that only about 80 per cent. of the sewage organic matter, after being discharged into river water, under properly controlled conditions, is completely oxidised by saprophytic bacteria to the end products, carbon dioxide, water, and ammonia. Of the remaining portions of the sewage organic matter, the larger part is converted into humus, and the rest into the other organic substances concerned in the life processes of the saprophytic bacteria, that effect the oxidation of the sewage matter.

For the examination of clean and polluted river waters, 500 to 100 c.c. of the sample, according to its organic content, are taken for an estimation, and a more dilute solution of bichromate, and smaller volumes of it and of acid than those employed in the above tests, should be used; but the concentration of the acid should not be reduced.

The authors have been induced to draw attention to the great value of acid

bichromate of potash in water analysis by a number of friends interested in the subject. Their experience with the use of this reagent leads them to express the hope that its use will become general in the analysis of sewage liquors and of river waters.

It is quite evident from the foregoing results, allowing for experimental errors, that it provides a valuable means for rapidly estimating the total organic content of a sewage liquor or of a river water, and also for testing the efficiency of any process in operation for the purification of sewage liquors.

No. 18.

ON THE RATE OF CARBOHYDRATE TRANSPORT IN THE GREATER YAM, *DIOSCOREA ALATA*, LINN.

By T. G. MASON, Sc.D.,

AND

C. J. LEWIN.

[Read February 23. Published separately February 23, 1926.]

Perhaps the most cogent argument against the phloem being the tissue normally responsible for the longitudinal transport of carbohydrates is its physical unsuitability for the transmission of solutions at high velocities. Dixon (3) was apparently the first to see this. His calculation of the rate of movement of the solution through the phloem of the rhizome supplying the potato tuber indicated rates as high as 50 cm. per hour. As a rate of this magnitude is physically impossible in the sieve-tubes, he suggested that the xylem was the tissue utilized for transport. In making his calculation he made two assumptions which are perhaps not justified. In the first place, it was assumed that the concentration of sugar was only 10 per cent., and in the second that a potato only 100 days old contained 24 per cent. of combustible matter.

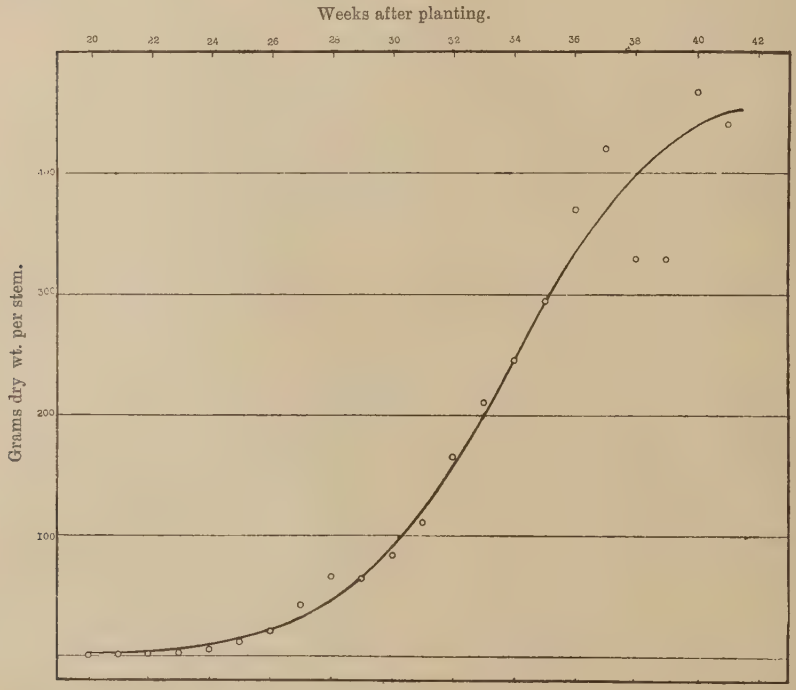
Curtis (2), and also apparently Artschwager (1), consider that Dixon's suggestion—that the xylem is better fitted than the phloem to carry solutes—does not simplify matters; chiefly, it would seem, because in the potato rhizome the xylem has an even smaller cross-section than the phloem. They apparently do not regard the open conduits of the xylem as better adapted than the colloid-filled phloem tubes for the movement of sugar. Curtis is therefore led to suggest that streaming and rotation of the protoplasm is the most likely explanation of the movement of solutes. His view apparently is that carbohydrate travels, not in solution, but either as sugar adsorbed on the colloids of the streaming protoplasm of the sieve-tubes, or as some condensation product of sugar.

The writers have recently made some observations on the movement of carbohydrates through the stem supplying the tubers of the Greater Yam. The work was carried out on the Moor Plantation, which is situated about three miles from the town of Ibadan, in Southern Nigeria. The tubers from 43 individual plants were dug and weighed every week, the dry weight being determined on only 6 tubers. As an individual plant may have more than one stem supplying its tuber (or tubers), the results have been expressed as the weight of dry material per single stem. For example, on the 17th of September (35 weeks after the date of planting) the average tuber-weight per plant amounted to 2,363 grams, while the average percentage of moisture in the tuber was 78·6, so that the average tuber dry weight was 505·7 grams per plant. As the average number of stems per plant at this time was 1·72, the dry weight per stem was evidently just 294 grams.

The results are shown graphically in the figure. The continuous line represents the curve of autocatalysis, and is calculated from the equation

$$\log \frac{x}{a - x} = K (t - t_1)$$

where x represents the mean dry weight of tuber per stem at any time, t, t_1 is the time at which half the final dry weight a is attained, and K is a constant. It will be noticed that the observed and computed values are in good agreement except towards the termination of the period of tuber development. There was difficulty at this time in obtaining a representative sample, owing to the limited number of individuals left growing.



Inasmuch as the stem had attained its maximum cross-section before the tuber began development, it will be evident that the results recorded graphically in the figure indicate the changes in the rate at which dry matter moved through the stem in the course of the tubers' development. It is interesting to note that active growth of the tuber did not begin until 28 weeks after the time of planting. Wet weight determinations of the stems and leaves (also of 43 individuals) showed an actual decrement in the thirtieth week, which continued throughout the period of the tubers' development until the dying down of the vine. The percentage of moisture in the tuber declined somewhat irregularly from 93 per cent. in the twentieth week to just 75 per cent. in the thirty-sixth week, after which there was little change.

The period of greatest activity in the development of the tuber, and therefore of sugar transport through the stem supplying it, occurred between the thirty-first and thirty-fifth week. The dry weight per stem increased during this period from 110 grams to 295 grams, so that there was a mean weekly increment of rather more than 45 grams. As just 95 per cent. of this is combustible, it would follow that 42·7 grams of combustible material were transported each week through the stem. Measurement on nine stems, collected at this period, gave a mean total cross-sectional area of sieve-tubes and vessels of 0·05747 ± ·0019 sq. cm. per stem; of this the sieve-tubes occupied less than 20 per cent., or 0·0115 sq. cm. If now it be assumed that the carbohydrates moved at a concentration of 25 per cent. in the sieve-tubes, a velocity of transport of $\frac{4 \times 42{\cdot}7}{7 \times 24 \times 0{\cdot}0115}$ or 88 cm. per hour would result. A movement at this rate is, of course, impossible through the sieve-tubes, even if their whole cross-section were available for transport. Thus, even when high concentrations are assumed, the phloem seems incapable of transmitting carbohydrates. That the phloem, in common with other living tissues, may transmit sugars at a limited rate is probably not in doubt, but that carbohydrates are *normally* transported through it seems impossible.

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No. 19.

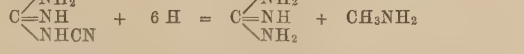
THE CONSTITUTION OF DICYANODIAMIDE, WITH A NOTE ON THE FORMATION OF A MERCURY DERIVATIVE.

By JAMES BELL, M.A., Sc.B.

[Read March 23. Published separately March 23, 1926.]

Dicyanodiamide, the di-polymer of cyanamide, was originally represented by a cyclic formula, two alternative forms of which are

The former of these is due to Hofmann, and the latter to Baumann. Bamberger, however, obtained guanidine and methylamine by the action of nascent hydrogen on this compound (Ber., 1883, **16**, p. 1459). This reaction indicated the presence of the ·CN radical in the dicyanodiamide molecule, and Bamberger, therefore, adopted the cyanguanidine formula, representing the reaction as follows:—



In a later paper (Ber., 1893, **26**, p. 1583) it was shown that hydrogen cyanide was first liberated, and then reduced to methylamine. Since then further experimental evidence has been brought forward in support of this view of the constitution of dicyanodiamide, and the cyanguanidine formula is now widely accepted by chemists. Several properties of the compound are, however, incompatible with this formula. A comparison with nitroguanidine suggests that cyanguanidine should have feeble basic properties. Nitroguanidine can form salts with acids, and, since the ·NO_2 radical is more electronegative than the ·CN radical, cyanguanidine ought also to behave as a base. Dicyanodiamide, however, behaves as a perfectly neutral body (N. Kameyama, J. Coll. Eng. Tokyo, 1922, **11**, p. 185), and this fact, together with the remarkable stability of the compound, is more in agreement with a cyclic structure than with the cyanguanidine formula.

Considerations such as these led to a re-examination of the experimental work on which Bamberger's conclusions were based. When dicyanodiamide in aqueous solution is boiled with dilute hydrochloric acid, hydrolysis takes place with the formation of dicyanodiamidine, or guanyl urea, according to the equation:—

$$C_2N_4H_4 + H_2O + HCl = C_2N_4H_6O \,.\, HCl.$$

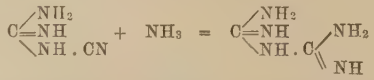
In Bamberger's experiments (*loc. cit.*) zinc and hydrochloric acid were added to a solution of dicyanodiamide, the containing flask being immersed in cold water. In this way, by not allowing the temperature to rise, he assumed that the formation of dicyanodiamidine would be prevented. This assumption, though not explicitly stated, may be gathered from the account of the work in question.

Experimental evidence is now brought forward to show that under the conditions of Bamberger's experiments, dicyanodiamidine is formed, and, as no quantitative results were given, there is no means of proving that the methylamine was derived from dicyanodiamide and not from dicyanodiamidine. Bamberger's evidence for the cyanguanidine formula is therefore inconclusive.

A more complete investigation of the action of nascent hydrogen on dicyanodiamide and on dicyanodiamidine has since been carried out by E. A. Werner and W. E. Holmes, whose results are not yet published, and to whom the discussion of the constitution of dicyanodiamidine and of the hydrolysis of dicyanodiamide is, therefore, left.

Other reactions have been brought forward as affording evidence in favour of the cyanguanidine formula. When an alcoholic solution of dicyanodiamide and ammonium chloride is heated in a sealed tube for 8 hours at 105° C., biguanide is formed (Smolka and Friedreich, Monatsh., 1888, **9**, p. 228). This compound may also be obtained by fusing a mixture of dicyanodiamide and ammonium nitrate or chloride (Davis, Jour. Amer. Chem. Soc., 1921, **43**, p. 2234).

The reaction is usually represented thus :—



A similar change takes place in the case of piperidine, copper piperylbiguanide, $Cu[NH:C(:NH).NH.C(:NH).NH.C_5NH_{10}]_2$, being prepared by heating dicyanodiamide, copper sulphate, piperidine, and a little water for some hours at 100–120° C. (Bamberger and Seeberger, Ber., 1891, **24**, p. 899).

While reasons for doubting the above simple explanation of the formation of biguanide are given by Werner and Bell (J. C. S., 1920, **117**, p. 1133, and 1922, **121**, p. 1791), it can be shown that, even if established, this explanation does not necessarily prove the cyanguanidine formula.

In considering these reactions, it should be made clear that the only difference between the Baumann and Bamberger formulae consists in the position of one hydrogen atom. While there are many examples of the ready mobility of the hydrogen atom in molecules, the fact that dicyanodiamide gives a neutral solution with water suggests that, under these conditions at any rate, there is no migration of this hydrogen atom to form cyanguanidine. However, under more severe conditions, it is possible that this migration would take place with the opening of the cyclic formula as follows :—

It is highly significant, therefore, that the above reactions, used to prove the Bamberger formula, take place only on prolonged heating at high temperatures, thus indicating that the first step in the formation of biguanide is this change to cyanguanidine. The reactions in question do not, therefore, disprove the cyclic formula.

Experimental.

In Bamberger's experiment, 1·5g. dicyanodiamide was dissolved in 45 c.c. water, and 9g. zinc and 15g. hydrochloric acid (38 per cent.) were added. As the acid was used up by the zinc, further quantities were added at intervals, the experiment lasting five days. The hydrolysis of dicyanodiamide in this case would be approximately the same as in a solution of the same strength, containing half this amount of acid, and allowed to stand for the same time.

The changes produced in a solution of this strength were, therefore, investigated. After remaining for 24 hours at ordinary temperature, the presence of dicyanodiamidine was proved by the formation of its characteristic copper derivative. Since dicyanodiamidine is a fairly strong base, and combines with hydrochloric acid, it could be estimated by the diminution in the amount of free acid present. Hence, by titrating a known volume of the solution at intervals with normal sodium hydroxide solution, using methyl orange as an indicator, the extent of hydrolysis was determined. The following results were obtained:—

Time.	Dicyanodiamide hydrolysed.
1 day	14 per cent.
2 days	24 ,, ,,
3 ,,	33 ,, ,,
4 ,,	40 ,, ,,
5 ,,	47 ,, ,,

When an $\frac{N}{5}$ solution of dicyanodiamide, containing one equivalent of hydrochloric acid, was allowed to remain at ordinary temperature, hydrolysis took place to the following extent:—

Time.	Dicyanodiamide hydrolysed.	Time.	Dicyanodiamide hydrolysed.
6 days	13·5 per cent.	24 days	41·0 per cent.
12 ,,	25·5 ,, ,,	30 ,,	45·3 ,, ,,
15 ,,	31·2 ,, ,,	36 ,,	48·3 ,, ,,
19 ,,	36·5 ,, ,,		

Conclusion.

The general properties of dicyanodiamide suggest that it is a cyclic compound rather than cyanguanidine, as proposed by Bamberger. Reasons are given for rejecting as inconclusive the evidence, on which Bamberger has based this view of its constitution. It is also shown that the formation of biguanide and its derivatives is not incompatible with the cyclic formula, and that these reactions do not, therefore, prove the cyanguanidine structure.

Note on a Mercury Derivative of Dicyanodiamide.

The only metallic derivatives of dicyanodiamide hitherto recorded are those of sodium and silver. Evidence is now brought forward of the formation of a mercury derivative, which can be obtained as a white insoluble solid by adding aqueous solutions of mercuric chloride and sodium hydroxide to a solution of dicyanodiamide. An investigation of this reaction showed that it was represented by the following equation:—

$$C_2N_4H_4 + 2HgCl_2 + 4NaOH = C_2N_4H_2Hg_2(OH)_2 + 4NaCl + 2H_2O.$$

Experimental.

By comparing the yields of the insoluble derivative, obtained by using varying proportions of mercuric chloride, the reaction was shown to involve two molecular proportions of the latter substance. With more than this amount of mercuric chloride, mercuric oxide was formed as well as the dicyanodiamide derivative. When two molecular proportions of mercuric chloride and a known excess of normal sodium hydroxide solution were used in the preparation of this compound, an examination of the filtrate showed that four molecular

proportions of sodium hydroxide had been used in the reaction, being converted to sodium chloride. All the chlorine present as mercuric chloride was accounted for in this way, and confirmation was given by the fact that the mercury compound in question contained no chlorine. These results afforded conclusive evidence for the above equation.

An analysis of the compound gave Hg = 77·07 per cent. $C_2N_4H_2Hg_2(OH)_2$ requires Hg = 76·35 per cent. The presence of two hydroxyl groups was confirmed by the evolution of water on heating the solid to 150° C., in accordance with the equation:—

$$C_2N_4H_2Hg_2(OH)_2 = C_2N_4H_2Hg_2O + H_2O.$$

The loss of weight found = 3·84 per cent. Loss of weight calculated from the equation = 3·85 per cent.

The author gratefully acknowledges his indebtedness to Professor E. A. Werner, Sc.D., for assistance given in the course of this work.

No. 20.

ON THE RATE AND MECHANISM OF THE AERATION OF WATER UNDER OPEN-AIR CONDITIONS.

By W. E. ADENEY, D.Sc., F.R.C.Sc.I., F.I.C.

[Read March 23. Published separately April 1, 1926.]

The problems of the rate at which the solution of oxygen and nitrogen occurs at the exposed surfaces of waters under open-air conditions, and of the mechanism by which the dissolved gases are transmitted to the unexposed portions of such waters, have become of considerable technical importance, especially in connection with the disposal of normal sewage liquors by dilution with clean river waters, as recommended by the Royal Commission on Sewage Disposal in its eighth Report (1912), and also in connection with the economical operation of the Bio-Aeration method of sewage purification.

A large number of experiments have been made in connection with these problems—first by the writer (1), then by him in collaboration with the late Mr. H. G. Becker (2), and latterly by him in collaboration with Dr. A. G. G. Leonard and Miss A. Richardson (3).

Solution of Gases by Well-Mixed Water in Thin Films.

Adeney and Becker employed a method for determining the rates of solution of oxygen and nitrogen from the air by water, every part of which is successively exposed in thin films, and becomes aerated independently of downward "streaming."

The method consists of enclosing a large bubble of air of known volume in a long narrow tube containing de-aerated water, also of known volume, and of allowing the bubble to ascend through the water column repeatedly until saturation of the water by air has been reached, the water-air surface remaining unbroken throughout the experiment.

The time taken by the bubble to ascend the tube was measured in every case, and found to be practically constant, at 18 seconds per double journey.

The loss of pressure in the bubble of air was measured, after each double passage up the tube, by means of a water manometer, and afforded the necessary data for calculating the amount of gas dissolved, step by step, until saturation was reached.

The air in the bubble was renewed after each observation, by taking out the stopper of the tube and inserting into the air-space a tube connected with a filter-pump, which drew a current of air through the space.

As the bubble of air ascends the tube from the bottom, the whole of the water gradually and uniformly flows over it in thin films, the mass of the water forming the films becoming mechanically and intimately mixed, after exposure

of their surfaces to the air in the bubble, with the result that the column of water, when the experiment is properly carried out, becomes gradually charged with small additions of air throughout its length, by repeated passages of the bubble along the tube, until saturation is reached. So that the air-content of the column of water is uniform throughout its length at any intermediate stage of solution up to saturation. Bubbles of air of 5, 7·5, 10, 12·5, and 15 c.c. in volume were employed.

In the case of the bubble of 15 c.c. in volume, the thickness of the film was found to be 0·05 cm., and its effective area 71·33 sq. cm.

The solution of oxygen and nitrogen was found to take place in accordance with the formula

$$w = w_1 - (w_1 - w_0)\, e^{-f\frac{a}{v}t}.$$

in which w = concentration at the end of t seconds.

w_1 = saturation concentration at the given temperature and pressure.

w_0 = initial concentration at start, when $t = 0$.

f = coefficient of escape of the gas from the water per unit area.

v = volume of water.

a = area of surface exposed.

The coefficient f varies with temperature according to the equations

Oxygen	$f = 0{\cdot}0096\ (T - 237)$	per second.
Nitrogen	$f = 0{\cdot}0103\ (T - 240)$	„ „
Air	$f = 0{\cdot}0099\ (T - 239)$	„ „

It is noteworthy that, when the rates of solution are expressed in percentages of saturation, the curves drawn from the percentages of saturation for the three gases lie very close to each other, those for oxygen and nitrogen (pure) being practically identical.

It is possible to calculate, with the aid of this formula, the rate of solution of oxygen or nitrogen from the air, by films of fresh or salt waters, under different conditions of area of film and total volume of water, or initial degree of saturation, provided that the calculations are based upon data derived from experiments carried out so that all of the water is successively exposed in thin films, and thorough mixing is thus ensured.

From the results of the experiments described in the papers, Nos. 31 and 44 (2), and with the aid of the above formula, the rate of solution of oxygen from the air by films of fresh and of sea water, 0·05 cm. thick, have been calculated for temperatures between 0° C. and 30° C. The values obtained are given in Table I.

TABLE 1.—Rates of Solution of Oxygen, expressed in Percentages of Saturation, by Films of De-aerated, Fresh, or Salt, Waters, 0·05 cm. thick, when uniformly exposed to the Air, *and independent of Evaporation and downward "Streaming."*

Temperature	0°	5°	10°	15°	20°	25°	30° C.
Percentage of saturation.	Time in seconds.						
10 ...	1·0	0·8	0·8	0·7	0·7	0·6	0·5
20 ...	2·1	1·8	1·6	1·4	1·3	1·3	1·2
30 ...	3·2	2·7	2·3	2·0	1·8	1·7	1·6
40 ...	4·5	4·0	3·6	3·3	2·9	2·7	2·4
50 ...	6·0	5·2	4·6	4·2	3·9	3·6	3·3
60 ...	8·0	7·2	6·4	5·7	5·0	4·6	4·3
70 ...	10·5	9·3	8·2	7·3	6·7	6·1	5·8
80 ...	14·0	12·5	11·0	10·0	9·0	8·3	7·7
90 ...	19·8	17·5	15·6	14·2	13·0	11·9	10·8
99 ...	39·6	35·0	31·2	28·4	26·0	23·8	21·6

Pressure, 760 mm. (moist).

For practical purposes these values may be taken as approximately representing the maximum rates of solution of oxygen from the air by the exposed surfaces of water exposed to open-air conditions, at the given temperatures, independently of evaporation and downward "streaming."

Solution of Gases by Quiescent Columns of Water.

In the case of quiescent columns of de-aerated water, freely exposed to the air, the dissolved atmospheric gases do not remain concentrated at the surface film. The latter, on the contrary, as the gases are dissolved by it, sinks towards the bottom of the column, for the reasons given below, with the result that the column becomes aerated gradually, and more or less uniformly, from top to bottom during every step of the process of aeration, until completely aerated. At no time does the surface layer of a column of de-aerated water become saturated until the whole column of water throughout its depth is also nearly saturated.

From the results of the writer's experiments with three- and six-foot columns of water, and from those of the experiments recently carried out with great care in collaboration with Dr. A. G. G. Leonard and Miss Richardson with ten-foot columns of distilled water and of solutions of sodium chloride of various concentrations, it has been established that the aeration of quiescent columns of de-aerated water, both fresh and salt, under open-air conditions, is to be ascribed largely, though possibly not wholly, to the effects of evaporation from the exposed surfaces of the water.

Apparently, as evaporation proceeds from the surface of a body of quiescent water, the density of the exposed surface film of water is increased relatively to that of the unexposed portions of the water as a result of cooling, and also, in the case of salt waters, of concentration of the salts in solution, and the denser water sinks in minute imperceptible streamlets towards the bottom. The continuous downward "streaming" thus produced causes a corresponding

upward displacement of unexposed portions of the water, and sets up an imperceptible, but efficient, process of mechanical mixing of the aerated surface film with the unexposed portions of the water. Although the mass of the water "streaming" downward from the exposed surface at any particular time must be relatively minute, it passes vertically downwards through the mass of water below the surface towards the bottom, and sets up a very efficient process of mixing, especially in the case of salt water. And since the experiments referred to above prove that the downward "streaming" is effective in causing as uniform mechanical mixing to depths of 10 feet as to 1, 3, and 5 feet, it may safely be assumed that it is effective in producing a similarly uniform mixing down to much greater depths.

The "streaming" sets up a more rapid and uniform process of mixing in salt waters than in fresh waters when the conditions obtaining over their exposed surfaces are favourable for free evaporation, owing, no doubt, largely, if not wholly, to the fact that in addition to the cooling effect of evaporation at the exposed surface film, there is also that of concentration of the salts in solution in the case of the former class of waters.

It may be noted, furthermore, that the process of "streaming" also proceeds more rapidly and uniformly at temperatures at and above 10° C. than below it in both fresh and salt waters. This is to be expected from the slow rate of evaporation at the lower temperatures, and from the fact of fresh water attaining its maximum density at 4° C.

It also depends upon the concentration of the salts in solution. The optimum concentration is equivalent to about 1·5 per cent. sodium chloride.

It may finally be concluded that there are five conditions upon which the mechanism of the aeration of fresh and salt waters, under open-air conditions, primarily depends. They are:—

(1) The area of water exposed to the air;
(2) temperature;
(3) humidity of the air;
(4) salinity (in the case of tidal waters);
(5) wind, causing both an increase in area exposed and an acceleration of evaporation per unit area.

The influence of the humidity of the air and of the salinity of the water upon the rate of "streaming," and therefore upon the rate of mixing of the exposed with the unexposed portions of a water, has recently been quite independently confirmed by Dr. J. A. N. Friend's interesting and important experiments on the corrosion of iron (4).

The area of open waters exposed, in common with the other conditions, will not remain constant, but will be liable to considerable variation, according to the force of the wind or of water currents, or of both.

The turbulent action of these two factors will also effect a more or less rapid increase in the rate of solution of atmospheric gases by the exposed surface films of the water.

The formula applicable to the calculation of the rate of aeration of wholly or partly de-aerated quiescent columns of fresh and of sea waters, when their exposed surfaces are undisturbed, and the humidity and temperature of the air are uniform, is of the same form as that above given for the rate of solution of oxygen and nitrogen by thin films of water, when independent of evaporation and downward "streaming" (2, **16**, 13).

The effect of the humidity of the air in contact with the water on the rate of aeration is shown by the following experiment:—

Four tubes, each 320 mm. long and 40 mm. diameter, were placed in a thermostat after being filled with de-aerated water. The upper ends of three of these tubes, after 50 c.c. water had been withdrawn from each, were connected in series by means of rubber corks and glass tubing, so that a current of air could be drawn through the air-spaces. The fourth tube was left open to the atmosphere.

Unfiltered air was drawn through the air-space of tube 1, then through two U-tubes containing calcium chloride, and thence into the air-space of tube 2, whence it passed directly into the air-space of tube 3. Thus the air was much drier when passing through the air-space of tube 2 than when passing through the air-space of tube 1; while the air on being drawn through the air-space in tube 3 was nearly, if not wholly, saturated with aqueous vapour.

Tube 4 was exposed to the open air in the middle of the thermostat, where the humidity of the air was considerable.

The results obtained from this experiment are given in Table 2.

TABLE 2.—Experiment showing the Effect of the Humidity of the Air on the Rate of Aeration of Quiescent Columns of Distilled Water. Temperature, 15° C.

No. of tube.	Depth of water.	Degree of aeration in p.c. of saturation.[1]	Value of f_1.	Time of Exposure. Actual.	Time of Exposure. Calculated from rate of solution at surface at 15° C. (Table 1.)
	cms.		per hour.	hours.	minutes.
1	27·07	41·5	0·34	43·0	22·8
2	26·37	63·2	0·61	44·0	46·9
3	26·69	31·3	0·23	44·5	10·9
4	30·20	32·7	0·29	42·0	13·6

Area of surface of column 1 = 12·56 sq. cms., of each of the other columns = 12·25 sq. cms.

f_1 = coefficient of escape per unit area.

On examining these results it will be noticed that the column exposed to the dried air absorbed more than twice as much air as the column exposed to the moist air.

It may be noted, in connection with the above experiment, that the values of f_1 in other experiments with quiescent columns of fresh, and of sea waters, when exposed to fresh air and kept at as uniform a temperature as possible, were found to be equal to 0·388 and 0·509 respectively.

The figures in the last column of the above table give the times in which the columns would have been aerated to the percentages of saturation which they respectively attained under the conditions of the experiment, had the aeration of each column proceeded at the rate given in Table 1 for surface films ·05 cm. thick, at 15° C.; that is, at the approximately maximum rate of solution of oxygen from the air by fresh and salt waters, as found by the bubble method for the same temperature.

[1] Initial aeration of each column = 10·3 per cent. saturation.

On comparing these figures with the actual times of exposure given in the table, it appears that the rates of aeration of quiescent columns of water by "streaming," under the specified conditions, were about 113, 56, 245, and 185 times slower than the initial rate of solution by the surface film, ·05 cm. thick, according to the humidity of the air passing over their exposed surfaces.

These rates of aeration apply only in cases of experiments with water columns in the laboratory, in which the surface layers can be preserved undisturbed without difficulty.

Under natural conditions, however, the exposed surface of open water is usually in a more or less disturbed condition, and then the aeration by "streaming" becomes greatly accelerated, as shown by the following experiment, in which the exposed surfaces of quiescent columns of de-aerated fresh and sea waters were kept in a continuous state of gentle disturbance. In this experiment two tubes, each 110 cm. long and 5 cm. diameter, were nearly filled, one with de-aerated fresh water, and the other with de-aerated sea water. A fine glass rod stirrer, bent into the shape of a gridiron, 4 cm. broad, was fixed so that its lower end dipped 2·5 cm. below the surface of the water in each tube, and was mechanically rotated slowly for 19 hours, the speed being such that the surface was only moderately ruffled. At the end of this time the stirrers were stopped, and samples were drawn from the top and bottom layers of the water column in each tube, and the dissolved nitrogen content was determined, with the following results :—

TABLE 3.

	Sea water.		Fresh water.		
	Top layer.	Bottom layer.	Top layer.	Bottom layer.	
At commencement ...	10·7	10·7	9·2	9·2	per cent. saturation.
19 hours later ...	98·3	96·5	80·4	73·4	per cent. saturation.

Temperature of each column, 8·3° C.

It is evident from the above results that, when the surface of the water is kept continuously and gently disturbed, the effective area exposed to the air becomes so much increased that the downward "streaming" is greatly augmented, with a corresponding acceleration of the rate of aeration of the whole column. And this is true when the experiment is carried out at the low temperature of 8·3° C. The results recorded in the table show that in the course of 19 hours the average aeration of the sea- and fresh-water columns was increased, on an average, from 10·7 and 9·2 to 97·4 and 76·9 per cent. saturation respectively.

These columns would have reached like degrees of partial aeration in approximately 13 and 5·5 hours respectively had their exposed surfaces remained undisturbed, and had the aeration of the columns proceeded at the initial rates of solution of oxygen as found by the bubble experiments.

It may, therefore, be fairly assumed that the minimum rates of aeration of open waters by "streaming" may be approximately twofold and fourfold slower respectively than the rate that would be found by calculation for the given depth from the results of the bubble experiments.

These rates of aeration for open waters may fairly be regarded as minimum rates, since the temperature at which the experiment was carried out was below 10° C., and since the air immediately above the columns of water must have been well charged with aqueous vapour during the experiment, owing to the

fact that their surfaces were exposed to air partially enclosed within the mouths of the tubes containing the water.

In the Bio-aeration method of purification the sewage liquid is intimately mixed, while in the aeration tank, by blowing air through it from the bottom of the tank, or by a propeller partially submerged in it. Whichever means of mixing is adopted, the surface of the sewage liquid is kept in a state of turbulent agitation, and it will probably be found on investigation that the minimum rate of aeration of the sewage liquid by downward "streaming" will be fully equal to, or, owing to the greatly increased surface area, may even exceed, that found by calculation from the results obtained by the bubble experiments for the given depth of the liquid, assuming an effective absorbing area equal to the undisturbed surface area of the tank.

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No. 21.

THE PENTOSAN THEORY OF COLD-RESISTANCE APPLIED TO CONIFERS.

By JOSEPH DOYLE,
Professor of Botany, University College, Dublin,

AND

PHYLLIS CLINCH, M.Sc.,
Demonstrator in Botany, University College, Dublin.

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General Introduction.

In spite of their great importance, whether we consider them geographically, as a unit of vegetation, or from the economic point of view, the Conifers have suffered comparative neglect from physiologists. In numerous articles that deal with winter-green plants, one or two Conifers will be, perhaps, found included, but it is impossible to determine from the literature whether the needle-leaved Conifers, at any rate, can be properly classed with the broad-leaved forms. They may be justly so classed, physiologically, but they, so far, have seldom received any such separate treatment as their importance would seem to deserve. A few examples will bring out the point. Within the group itself the literature has been searched in vain for any differential consideration of Conifers as members of the sclerophyllous flora of the Mediterranean type, and as members of the Northern cold temperate taiga, although the physiological conditions must be very different in the two regions. Work on rates of assimilation and respiration are scanty in the extreme. From only two aspects, indeed, has the group received any adequate examination; namely, the water relations of some of the forms and the changes that take place in the storage reserves at various seasons of the year. This latter point has largely been dealt with for the Conifers in company with the broad-leaved evergreens, but, even here, work has only progressed sufficiently far to bring out the difficulties. Thus, if we consider the question of the changes taking place in the leaves, since this is the point in which they differ from deciduous forms, we find that Lidforss (14, 15) claims that all winter-green leaves are starch free during the cold period. Even in Padua he reports the same phenomenon, although the average temperature there is much higher than in his native Sweden. In his northern work he only includes a few Conifers, at Padua the only Conifer examined was *Juniperus communis*. We may remark here that Juniperus proved an exception to the other Padua forms, containing quite a supply of starch in the mesophyll in the first week in January, when the examination was made. He deems this a response to the growth of the northern form in a region of higher winter temperature. But he did not examine Juniperus in his Swedish work, or he could readily have found that Juniperus, and perhaps others, seem to have a different carbohydrate balance from the Pines and their allies, a fact which we hope to deal with later.

He further claims that the starch changes to sugar, remaining in the leaf. Although he carried out no quantitative work, it may be noted that such an increase in sugar in winter-green leaves during the cold period has been substantiated by many subsequent workers of which it suffices to name Lewis and Tuttle (13), and Michel-Durand (18). To what extent the principle of sugar increase in winter-green leaves in the cold season is applicable, is not yet determined, nor what degree of cold is necessary to bring about such a change from starch to sugar. Thus, Miyake (19), in a survey which covered nearly every winter-green shrub and tree in Japan, including about twenty Conifers, finds great variation in the retention of starch in different species even in the same locality. *Podocarpus nageia* at Tokyo has its mesophyll completely free from starch in mid-winter, while *Podocarpus macrophylla* retains a moderate amount of starch even during the coldest period. The same phenomenon appears in the Conifers grown at the Botanical Gardens, Glasnevin, Dublin. Miyake, however, further concludes that in the colder parts of the Japanese islands, the starch almost completely disappears. Yet we find that, in, say *Pinus thunbergii,* Miyake reports quite a moderate development of starch in trees growing in Kumamoto, while the same species at Dublin, enjoying a slightly higher temperature, is entirely starch free. In Japan this species only remains free from starch in the cold areas of Sapporo, where the temperature averages several degrees below freezing for two or three months, nearly ten degrees below the Dublin station. In fact the whole situation needs a thorough examination of the factors determining the retention or dissolution of starch in Conifer leaves during the winter months. The same uncertainty applies to the development of oil or fat in Conifer leaves in winter. Such a development has been often described in the past, as usual mostly for broad-leaved winter-greens, and has been more recently described by Tuttle (27, 28). In view of Fischer's well-known work on fat and starch trees, this phenomenon may be of great importance, but the whole situation is complicated by the claim of Meyer (17), who maintains, after a survey of the older literature, that the so-called fat, staining with osmic acid, is not fat at all, but a substance of unknown composition which does not rise or fall in amount with the seasons, but steadily accumulates with the ageing of the leaf. That Conifers may be included in this claim is shown by the similar behaviour of *Taxus baccata* and Ilex. Comparative quantitative work on the seasonal variations of starch and true fat seems completely lacking. In short, the seasonal changes in winter-green leaves are still not accurately known; in Conifers the question has received no special consideration, but a consensus of opinion seems to favour the general claim of, at any rate, an increase of sugar during the colder months, associated with a diminution of starch content. Intensive quantitative work is still largely lacking.

If we turn to a consideration of the regeneration of starch in those forms in which starch disappears from the leaves in winter, we find an equal uncertainty. Lidforss (14, 15) kept plants at a warm temperature in the dark, but in most cases there was no regeneration of starch except in the guard cells. In three forms, however, starch appeared in the mesophyll under such conditions. Tuttle (27) finds that starch is readily regenerated in winter in *Limnaea borealis* when that plant is kept at a warm temperature, but exposure to increased warmth of *Picea canadensis* for five weeks produced no effect. Thus temperature may be an important factor in some cases, but it is obviously not the only factor in others. Spoehr (24) seems to suggest that temperature is the determining factor, because he actually comments on Lidforss' work in the following words:—"That temperature is here actually the determining

factor is demonstrated by the fact that when the leaves during any time in winter are brought to a higher temperature, starch formation takes place very readily." Actually, as already stated, Lidforss found starch regeneration under such conditions an exceptional phenomenon. Only by wounding the leaf, in most cases, could such regeneration be obtained in the neighbourhood of the wound, an appearance explained by him on the assumption that the stomata of winter-green plants are hermetically sealed in winter, that oxygen is necessary for starch regeneration, and that puncturing the leaf provides the necessary oxygen, so that, even to Lidforss, temperature is only one factor. We certainly cannot yet say that it is the controlling factor. As Sinnott (23) points out, the winter changes in the stems of trees cannot be produced in summer by lowering of temperature, but develop in autumn, while the environment is usually far from harsh, not only in northern trees but in trees of the frostless areas of the Gulf States of America. Further, though Lidforss seems to feel that there is a minimum spring temperature below which starch regeneration is not possible — he even suggests 5° C. for the winter-green herb *Hologteum umbellatum*—yet Lewis and Tuttle report the appearance of starch grains in the leaves of Picea in the spring of northern Canada at a time when the thermometer was still reading 15° C. below freezing; while Winkler (quoted by Czapek (5)) finds that many plants can form starch from sugar at freezing point temperature. Czapek (5) himself, while inclining to the view that temperature is a determining factor, affirms that its action is such as to prevent the formation of starch sugar at low temperatures unless the sugar concentration is greatly increased. Sinnott (23) and Tuttle (27) have made suggestions as to enzyme activities. Work is at present in progress on the question of the enzyme condition in winter and summer of Conifer leaves, which, it is hoped, will yield some result of interest.

It is still entirely uncertain whether the starch which sometimes appears in small quantities in leaves in winter, and the starch which first appears in spring may not be photosynthetic starch. Miyake (19) is of that opinion, and Ehlers (8) has shown how the temperature of Pine leaves in the sun in winter may be much above the surrounding air. But this opens the almost untouched book of the photosynthetic capacity of Conifers, at any period of the year. Stalfelt (25) seems practically the only one who has dealt even with the general question. He deals with the rate of carbon assimilation of pine and spruce leaves in relation to temperature, light intensity, and CO_2 content of the air, and the contribution seems one of the very few noteworthy additions to our knowledge of the physiology of Conifers. He states, however, that there is little or no assimilation below about 5 per cent. of full sunlight. Now he has also claimed, as quoted by Stiles (26), that in the open air for all plants maximum assimilation is only reached with full sunlight, while Boysen-Jensen concludes that the maximum assimilation of plants growing under natural conditions is exhibited in light intensities well below that of sunlight, the maximum assimilation of forest trees being reached in an intensity of illumination of less than 5·5 per cent. of full daylight. In other words, not only are the special problems of the assimilation of Conifers untouched, but even the general questions relating to it have still to be answered.

We noticed earlier that Lidforss claims that the stomata of winter-green plants are hermetically sealed in the cold season. This is disputed by both Miyake and Ehlers, among others. But without further reference it suffices to say that we have no knowledge of any value on the physiology of the stomata of Conifers. The stomatal action in the flowering plants is still poorly understood, although there are some notable contributions in that field of

research, such as that of Loftfield (16) and others. But the only knowledge we have of the action of Conifer stomata is contained in the short articles of Neger (20), Dengler (7), and Weber (30), which are almost wholly concerned with methods of determining the degree of stomatal opening—a matter of some difficulty in Conifers—and hardly at all with the nature of their functioning. It needs no further elaboration to emphasize the enormous gap which such a situation presents in our knowledge of the physiology of the group.

Some attention has been paid to the water relations. Weaver and Mogenson (29) report that the transpiration of Conifer branches in winter is only a minute fraction of the summer rate, and no greater than that of the bare twigs of deciduous trees. To what extent that is relatable to the mid-winter evaporating power of the air, how far due to stomatal closure and leaf structure, and how far due to internal physiological changes in the leaf itself, has not been analysed. Bates (3), in a stimulating article full of detail, has dealt in general with the water requirements of some Rocky Mountain Conifers, especially during the growing season. This article is of the greatest interest, and brings out very powerfully the questions calling for solution. Korstian (12), dealing with sap densities in relation to environment, particularly cold and drought, includes details of many Conifers, but he, again, presents problems rather than solves them. Both Bates and Korstian relate sap density to drought- and cold-resistance, though there seems to be great disagreement between their data. Finally we may mention Pearson (21), who has described an object lesson in the resistance of Conifers to desiccation. He stopped watering potted plants of *Pinus scopulorum*, *Pseudotsuga taxifolia*, *Pinus aristata*, and *Picea Engelmanni* on August 20, the transpiration dropping to 3 per cent. within a month, when the soil was just at the wilting co-efficient. For two months transpiration was almost imperceptible. The plants were transferred to a greenhouse in October with increase of temperature, but with no ensuing increase of transpiration. On November 13 two of the yellow pines were watered. Transpiration jumped to the normal level, and the plants soon gave new shoots. The others stood all the winter, and not till April of the succeeding year were they listed as dead. Here again, then, we may say in general that the data presented in the few references quoted in relation to the water relations of Conifers demand much further experimentation, not only to solve the problems, but, in many cases, even to clarify the problems at issue.

This rapid survey of some points in Conifer physiology, in which merely a few representative citations have been made, might be extended indefinitely, but sufficient has been presented to bring out the points we wish to make—the subject of Conifer physiology is an open field.

Hardiness and Pentosans.

The data presented later deal with the Conifers as types of hardy vegetation, and as useful material to test the general applicability of a theory recently propounded to account for hardiness in plants. There is no need here to enter into the extensive literature of hardiness. The older contributions are well summarized in Pfeffer's Physiology, while complete summaries up to date are to be found in Chandler (4) and Rosa (22). It is obvious from such surveys that the factors making for cold-resistance are still obscure. The importance of the problem, however, in Agriculture and Forestry is shown by the large number of recent articles in the Journals, especially American and agricultural journals, in which contributions to its solution have been made;

but, in spite of such increasing volume of literature, it is of interest that, as late as 1921, Korstian (11) bemoans, in America, the almost total lack of knowledge of the frost-resistance of forest types in their natural habitat. The theories which have been proposed to account for killing by frost are fairly well known. It seems generally recognized that frost-resistance is protoplasmic, and that osmotic concentration as such, except in so far as it lowers the freezing point, is not important. That the seat of damage is the plasmatic membrane and not the whole body of the protoplasm has been urged. The nature of the damage has been claimed by Gorke and others to be an irreversible precipitation of the proteins, caused by the increased concentrations of the sap salts as the water leaves the vacuole, and not merely a mechanical injury due to the loss of water from the whole protoplasm or the membrane; while Harvey emphasizes the importance of increased acidity causing the protein precipitation, an increase which he claims is brought about by freezing. From among the theories proposed to account for frost resistance, mention may be made of two. Harvey claims a change in the proteins yielding an increase of amino-nitrogen, resulting in a lessened precipitation. Lidforss (15), impressed by the ubiquitous increase of winter sugar in winter-green leaves, claims the sugar increase as the protective device. Incidentally it is worthy of note that these two theories are not so mutually antagonistic as they first appear. Harvey, as we have seen, claims an increase in amino-nitrogen. Lidforss claims that the winter change in evergreens is two-fold, namely, a lessened protein synthesis, resulting in an increase in substances such as asparagine, which, even in vitro, increases the rate of starch hydrolysis, causing an increase in sugar. This substance, he finds, greatly lessens the precipitation of proteins during experimental freezing. So, both recognize the importance of amino-nitrogen.

Recently, however, Rosa (22), recognizing the unsatisfactory nature of so many contradictory hypotheses, has attempted to frame one which might serve better. In the first place, Rosa is impressed by the loss-of-water aspect of the freezing, feeling that even the precipitation of proteins, whether by salting out, by increased acidity, or other similar cause, is merely an expression of the disturbance of the colloidal water balance. Now, there is no doubt that the water balance is of importance. Both the older and the newer literature are full of references, for instance, to the greater tenderness of plant tissues with high water content. Johnson even makes water content his index of hardiness in peach buds. Strausbaugh has an interesting article in this connection, on dormancy and hardiness in plum buds. He finds that deeper dormancy accompanies greater hardiness and earlier maturity, and presents results to show that (1) drying out is slower during dormancy, (2) susceptibility to frost injury increases with the breaking of the dormant period, (3) the moisture content of hardy buds is fairly constant during dormancy, but that of semi-hardy buds varies with temperature, a lowering of temperature causing a decrease of moisture and vice versa, and (4) at low temperatures, therefore, the hardy buds may actually have a higher water content than the less hardy. He concludes that the real difference between the hardy and less hardy varieties is not merely a difference in water content, but a difference in relative water retaining capacity, and suggests that the change increasing the power of imbibition involves the proteins. Rosa, however, further impressed by the value of hydrophilous colloids, especially pentosans, in the hydration work of MacDougal and others on growth, succulence, imbibition, and similar phenomena, has based his theory of resistance on pentosans. He conceives the pentosans, owing to their great power of imbibition, as holding during freezing sufficient

water against the tendency towards ice formation to preserve intact the physico-chemical structure of the protoplasm. Unfortunately he has not followed the theory fully in many of its physico-chemical implications, but he has certainly produced considerable evidence in its favour. Experimenting with young cabbage plants subjected to various hardening treatments, seldom of greater duration than fourteen days, he finds increased pentosan content, lessened water loss by transpiration, decreased rate of dehydration, more water unfrozen in dilatometer measurements, and suitable changes of acidity– everything falls into a serried phalanx of support.

This hypothesis so strongly attracted us that it seemed advisable to seek for further support. Winter-green plants, and especially hardy winter-greens like the Conifers, should, it was thought, if the theory be sound, yield further evidence in its favour, above all if approached on a seasonal basis. Here we have the same organs, the leaves developing in the milder season, and yet remaining on the plant to face the rigours of the winter. Unless the slow autumnal hardening caused a different result from the rapid hardening of the cabbage, or unless the pentosan theory was of quite limited application in the plant kingdom, leaves of Conifers seemed good material for a test. The following work was, therefore, proceeded with.

Experimental Results.

A number of Conifers were accordingly examined throughout the year, attention being paid to the three following points:—

(1) The seasonal pentosan content.
(2) The seasonal changes in H-ion concentration.
(3) Comparative rates of dehydration in relation to their pentosan content.

The data relating to the first and second of these are here presented. The data relating to the dehydration rates it is hoped to present separately in a second communication.

Seasonal pentosan content of some Conifer leaves.

Work was confined to the leaves because, in the first place, direct comparison with Rosa's work on the leaves of the cabbage was desired, and, in the second place, because they are the more exposed organs on the plant, and last during the different seasons. The following Conifers were dealt with:—

Pinus montana v. *uncinata,* Willk. [Mountain Pine].
Pinus laricio v. *nigricans,* Parl. [Austrian Pine].
Picea sitchensis, Trautr. and Meyer [Sitka Spruce].
Pseudotsuga Douglasii, Carr [Douglas Fir].
Tsuga Albertiana, Sénécl. [Western Hemlock].
Abies pectinata, De C. [Silver Fir].
Cupressus Lawsoniana, Murr. [Lawson's Cypress].

and for certain comparative purposes—

Larix europaea [European Larch].
Fagus sylvatica [Beech].
Larix leptolepis [Japanese Larch].
Acer pseudoplatanus [Sycamore].

These were chosen in the main because they were all readily available, and were well-known important forms. Although all the Conifers used are about equally hardy under our Dublin conditions, the silver fir and the Douglas fir

seem more sensitive to spring frosts than the others. The Sitka spruce is also somewhat frost sensitive. The *Pinus montana* used, judged by all accounts, seems capable of standing exposure elsewhere more severe than any we here experience, without suffering any damage. Though not remarkably resistant to cold, the Austrian pine is still quite frost-hardy. Its inclusion here is due to its great drought-resisting powers. Cupressus has the reputation of being unaffected by the severest frost, and was at first chosen for comparison as one example outside the Abietineae. It was a lucky and interesting choice. The larches are representatives of deciduous Conifers, in which no preparation for winter is to be expected in the leaves. The beech and sycamore allowed of comparison with deciduous Conifers, these two being decided on because the beech has the reputation of being sensitive to spring frosts, while the sycamore is notoriously hardy. The material was secured largely from the Botanic Gardens, Glasnevin. The Forestry Station, Avondale, Co. Wicklow, supplied regularly *Pinus montana* and the Douglas Fir. One batch of *Abies pectinata* was secured from about 6,000 ft. in the Alps in mid-winter by the courtesy of the Forestry authorities in Zürich. In view of its habitat it was thought that it might show greater results from its severe hardening process than our local trees. Unfortunately it was not possible to secure a seasonal supply from the Alps, but a number of records throughout the year from spring to the succeeding January were made in the case of the other forms. To avoid any error due to deviation in content in different parts of the same tree—though at easy cutting level the variations here were very slight—or in different trees, the same trees were kept to throughout, and numerous small current-year shoots, from as many places as possible, were taken, and a few leaves used from each shoot. The work was thus confined to the current, one year, shoots from their opening in spring to the succeeding January. As the chemical estimations were slow, and as the time available was limited, this seemed the most satisfactory manner of reducing the possible error due to variations in parts of the trees. Immediately after gathering, the material was brought to the laboratory for estimation. This entailed only a short delay for the Glasnevin specimens, but a delay of several hours in the case of the Avondale ones. But, as we were unable to demonstrate any but the slightest variations from Glasnevin specimens collected either at different times of the day, or collected and retained for some time before proceeding to the estimations, we decided that any error due to the delay in reaching the laboratory of the Avondale specimens was within the limits of error of the method of analysis, which is notoriously inexact.

Before proceeding with the estimations a preliminary word on pentosans is desirable. As in the case of frost resistance there is no need to cover the older literature, as it is fully summarized in Czapek (6). Since then there has been no great addition to our knowledge of pentosans, either from the pure chemical aspect, or from the point of view of their function in the plant, except of course a realization of the importance of pentose sugars, and their derivatives, in relation to xerophytism. One point, however, deserves attention. The earlier workers, almost without exception, estimated in their various plant tissues the total pentosan content hydrolysable by fairly strong acid. Spoehr (24) pointed out, however, the probability that such a procedure included in one class pentosans intimately, and perhaps permanently, associated with the wall; and pentosans, much less closely bound to the wall, if at all, perhaps even associated with the protoplasm, and of active metabolic importance. The commonly recorded result of a relative increase of total pentosan with age he attributes to the common increase of wall substance with age. If

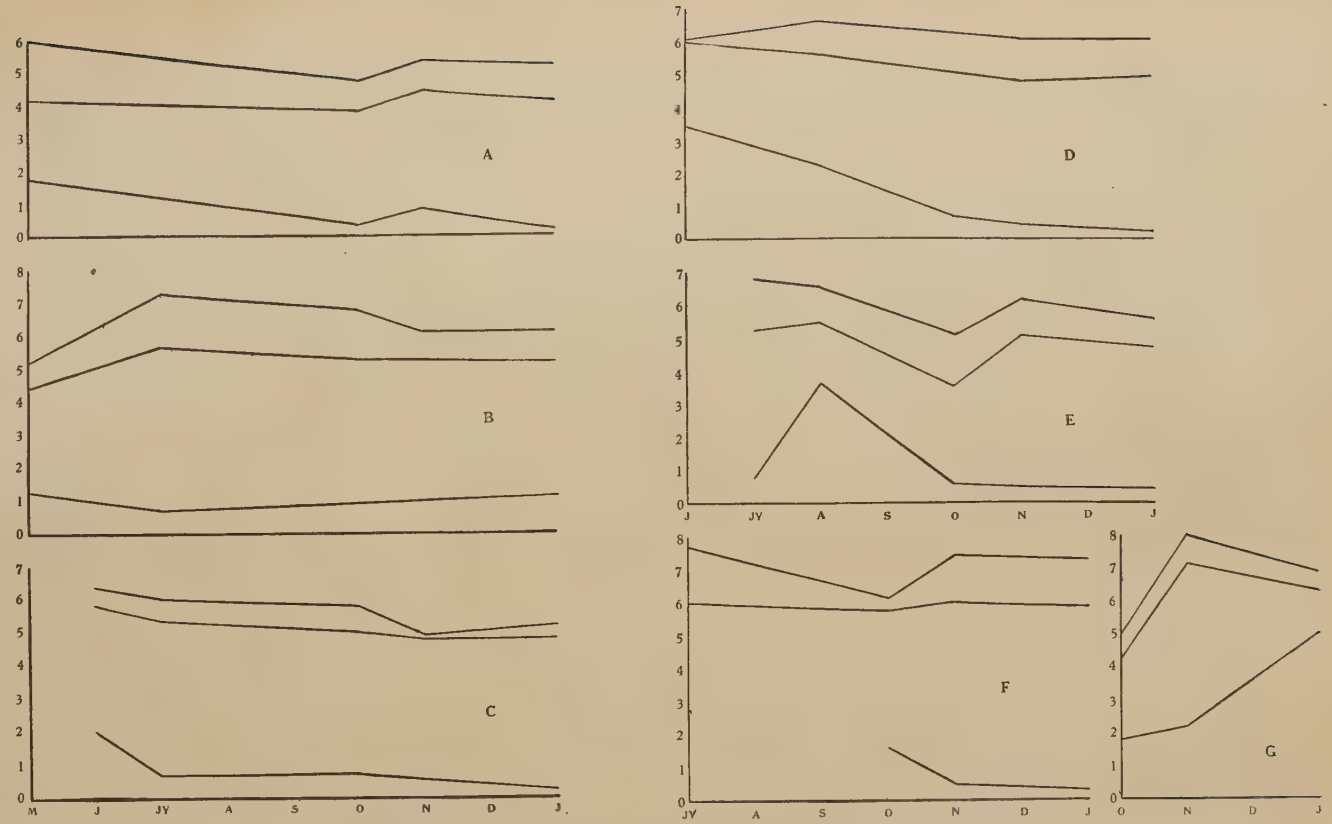
tissues are hydrolysed with weaker acid (he finds 1 per cent. HCl satisfactory), an end point is quickly reached. Subsequent hydrolysis with the customary stronger acid reveals a further pentosan content. Considering that the latter is due to wall breakdown, he limits himself to the pentosan yield estimated from a treatment of the tissue by 1 per cent. HCl. He finds then no definite increase with age, but if anything the reverse. Thus, joints of Opuntia of a certain age held 9·55 per cent. pentosan, while joints three years older contained only 6·70 per cent. He finds, too, great variation—from 1·38 per cent. to 12·17 per cent. in one case—throughout the year, a change bearing no relation to advancing age, but to the seasonal distribution of rain and temperature. Rosa (22) goes a step further, and, considering that those pentosans might be of greatest value to the plant as a defence against cold which were, owing to their hydrophilous nature, more or less water soluble, distinguishes further between pentosans extractable by hot water and those estimated from a 1 per cent. HCl hydrolysis of the residue. In fact his published figures show that the pentosan increase with hardening which he reports is due to the water-soluble fraction, the other changing but little. There are a few earlier accounts of these water-soluble pentosans in Czapek (6), but even now we know very little more about them chemically, than is expressed in his phrase:—"Doch ist über diese Wasserlöslichen Pentosane noch nichts bestimmtes in Erfahrung gebracht." However, in view of these results of Rosa, Spoehr, and others, we estimated separately (1) water-soluble pentosans, (2) pentosans subsequently extractable with 1 per cent. HCl, and (3) residue pentosans extractable only by 12 per cent. HCl. Seasonal records of water-soluble pentosans, and what we may call the 1 per cent.-soluble pentosans, seem few. Abbott (1) states that an increase of water-soluble pentosans takes place in shoots of apple and peach from July to October, but he has no estimations of the 1 per cent.-soluble group. Spoehr gives the seasonal variations of the 1 per cent. group only, while we have not yet met any examination of an evergreen plant, Conifer or Angiosperm along these lines. Even in an article on the pentosan distribution during growth of corn, published in 1923 (10), only the total pentosan content is recorded, and no attempt is made to analyse more closely its component fractions. We seem to find ourselves in the position of having no other corresponding work with which fully to compare our results.

For the pentosan estimations the ordinary furfural method was used. This has lately fallen into disfavour owing to its recognized lack of exactness. Spoehr, reviewing the work of Davis and Sawyer and other investigators, is particularly severe upon it. It is replaced by fermentation methods, whereby, after hydrolysis of the pentosans to pentose, any hexoses present are fermented away by yeast, and the residual pentose estimated by a copper method. But it is now known that the hexoses cannot be completely removed by simple fermentation by yeast. Their total removal is a matter of the gravest difficulty. What percentage error is involved being still a matter of individual investigation, it seemed to us that the older method would fill our needs as well. The error involved we admittedly assumed to be somewhat the same over all the readings, and, in any case, we felt that, until we had some idea of the quantities involved, and the possible seasonal variations, which should be very marked if support for Rosa's theory was to be forthcoming, the question of the relative error of the furfural and the yeast-copper method could await examination.

The leaves, as soon as possible after being gathered, were weighed in a weighing bottle, and then extracted in a Soxhlet apparatus over a water bath, with boiling 98 per cent. alcohol to which a few drops of ·88 ammonia was

added to neutralize plant acids. The extraction was slow, as it was not deemed complete till all the green colour was removed from the leaves. This usually took about 3 days. When the extraction was complete, the leaves were washed with alcohol, and the washings, added to the alcoholic extract, put aside. This alcoholic extract contained, among many other substances, the mono- and di-saccharides, but not the polysaccharides. The leaves were then thoroughly dried, cooled, weighed, and ground rapidly in a small mill of our own devising. In this material the pentosan present was estimated in three stages. The powdered leaf material is weighed, and extracted with distilled water over a bath for about five hours in a small flask fitted with a reflux condenser. After cooling it is filtered, and the residue on the filter is well washed. The filtrate contains *the water-soluble pentosans,* and is the first stage in the extraction. The amount of pentosan is determined, in the usual manner, by running 12 per cent. HCl into the filtrate, kept briskly boiling, in a distilling apparatus fitted with a dropping funnel, which allows the necessary frequent additions of 12 per cent. HCl. When the distillate no longer gives a red reaction with aniline acetate, the process is stopped: pure phloroglucine solution is added to the distillate, and the black precipitate which gathers over-night collected and weighed, and from it the pentosan content calculated by formula. The leaf residue is now subjected to a second extraction, using 1 per cent. HCl instead of distilled water. We must note here that, in preliminary manipulations, by stopping the extraction at intervals, filtering, testing the filtrate by distilling with 12 per cent. HCl as before, and continuing the extraction again with fresh 1 per cent. HCl, a definite end point was reached, usually after three hours. We agreed with Spoehr that 1 per cent. HCl was a suitable concentration, as stronger acid not only did not give so clean an end point, but also tended to a furfural formation. Weaker acid proved so slow in reaching an end point as to be tedious. In this way, then, the less firmly bound pentosans were hydrolysed to pentose, which was estimated in the filtrate in the same manner as before. This fraction we subsequently call the *1 per cent. HCl fraction.* The sum of these two we call the total of loosely combined pentosan or simply the *"total loose."* In some of the estimations it occasionally happened that the preliminary water extraction was omitted, the first extraction being done with 1 per cent. HCl. In such a case the value of pentosan resulting corresponded to the sum of the water-soluble and 1 per cent. HCl fractions, and is only recorded with the "total loose." In the third stage the leaf powder residue from the 1 per cent. HCl extraction is itself placed in the distilling flask and subjected to distillation with 12 per cent. HCl, exactly as in the other cases. This third estimation gives the amount of firmly combined pentosan, and is referred to as the *residue 12 per cent. HCl fraction.* As these quantities have to be calculated on the total dry weight of the leaves, the weight of the soluble material in the alcoholic extract had to be accounted for, by distilling off the alcohol in a small tared flask, subsequently carefully dried in the oven and the weight of the residue calculated. The weight of this residue, plus the weight of the dried leaves after extraction with alcohol, gives the total dry weight of the leaves, and on this the results are calculated.

The results of the estimations are recorded most conveniently by the series of graphs. The totals are of about the same magnitude as those of Rosa, whose figures for total pentosan lie between 3 per cent. and 6 per cent. on a dry weight basis, percentages which correspond with those outlined by the middle curve in each case. We rather expected much higher values, especially for the winter pentosan content of such hardy forms as the Conifers. Before commenting on the graphs, in which the seasonal variations are shown, it is



Graphical record of the seasonal pentosan distribution in the Conifers examined:—

A, Picea; B, Abies; C, Tsuga; D, Pseudotsuga; E, *Pinus montana*; F, *Pinus laricio*; G, Cupressus.

In each case the lower line is water-soluble pentosan; the upper line is the total of all pentosans present; and the middle line the total of loosely combined pentosans. The ordinates are per cent. pentosan.

necessary to tabulate a few isolated records which were made, and which will be referred to later.

Table 1.

Pentosan Content of Larches, Beech, and Sycamore.

Type.	Month.	Water soluble.	1 p.c. HCl soluble.	T.L.	Residue 12 p.c. HCl soluble.	Total.
Larix leptolepis ...	May	·79	3·02	3·81	Nil.	3·81
„ *europea* ...	„	·66	2·24	2·9	Nil.	2·9
„ „ ...	Oct.	·36	3·02	3·38	·55	3·93
Beech	June	·63	8·31	8·94	1·3	10·24
Sycamore ...	„	·86	3·47	4·33	2·21	6·54

In this table and the succeeding one the column T.L. is the sum of the water-soluble pentosans and those subsequently extractable by 1 per cent. HCl. The sum is referred to as the total of loosely combined pentosan, in contrast to that residue only extractable by 12 per cent. HCl.

In a few cases, as a result of preliminary examinations, estimations are available for two successive winter periods. As these are leaves of the same relative age, but which have been hardened by different autumnal conditions, the agreement in some cases is surprisingly close. In the preliminary ones no estimation had been made of water-soluble pentosans before proceeding to the 1 per cent. HCl hydrolysis. In the later ones, therefore, only the total of loosely combined pentosan is included, no distinction being drawn between the W.S. and 1 per cent. HCl fractions.

Table 2.

Comparison in Successive Winter Periods.

		T.L.	Residue soluble in 12 p.c. HCl.	Total.
Abies pectinata	I ...	5·14	·70	5·84
„ „	II ...	5·15	·94	6·09
Austrian Pine	I ...	5·93	1·43	7·36
„ „	II ...	5·93	1·34	7·27
Sitka Spruce	I ...	4·17	1·22	5·34
„ „	II ...	4·10	1·16	5·26
Douglas Fir	I ...	3·57	·49	4·06
„ „	II ...	4·85	·70	5·55

The variation in the Douglas Fir makes the agreement in the case of the other three forms all the more remarkable, especially in the loosely combined quota. It may be a mere chance, but until the point is re-examined more closely we only wish here to call attention to it.

Turning to the graphs, we can examine the seasonal distribution of the pentosan classes. In each case the lower line represents the water-soluble pentosans, the middle line the total loosely combined, the upper line the sum total of all. The difference between the lower two gives the fraction soluble in 1 per cent. HCl; the difference between the top and middle graphs gives the residue only extractable by 12 per cent. HCl. The late unfolding of the leaves

accounts for the first reading for the Austrian Pine not being available till July. By an oversight the water-soluble fraction was not separately estimated till October. Cupressus was not touched in any way till October. In a few other cases discrepancies in duplicate results necessitated the discarding of occasional readings, but there is still abundant evidence to test the soundness of Rosa's theory as applied to Conifers.

Now, as the "total loose" comprises the main bulk of the pentosan content, analysis of the two lower curves should yield support to Rosa if any is to be forthcoming. But it certainly does not seem possible to produce that support. Thus, looking at the graphs of water-soluble pentosan, we find, with the striking exception of Cupressus, that the period for which cold-resistance is presumably most necessary is characterized by a minimum value. Certainly Cupressus is frost-hardy in these islands, but is *Pinus montana* v. *uncinata* any less hardy? Again, with the exception of *Pinus montana,* those forms in which an estimation of their water-soluble pentosans is available for the first month of their growth show a marked maximum for this period, which is just the period when they are most sensitive to frost. The sudden rise in mid-summer in *Pinus montana,* a rise which is definitely substantiated, seems rather to indicate that we are dealing with some, as yet, obscure phase of the carbohydrate economy of the plants in question, each plant following its own lines, which may greatly vary.

A consideration of the pentosan content hydrolysable by 1 per cent. HCl obviously brings us to the same conclusion. Irrespective of the monthly variations, the curves show that, as far as the data are available, the January content is practically the same as the content of the previous July; while in Cupressus, though the earlier figures are not available, the drop to so much lower a content in January prevents us making use of the fact that there is an average rise from the early leaf stage to winter maturity. Further, if the 1 per cent. HCl is of importance, the young Beech leaves should be much the hardiest of all the forms. This is certainly not so. Apart from the Conifers, the young Sycamore is definitely much more hardy than the Beech, and yet the latter contains on the average 2½ times as much of the 1 per cent. HCl quota as the former. There seems, however, to be some relationship between the higher spring content of water-soluble pentosan of some of the forms and a high water content. Thus the young leaves of the Douglas Fir, standing at an average water content of 74 per cent., drop to 60 per cent. in a month or so; Tsuga drops from 71 per cent. to 59 per cent.; *Picea* from 78 per cent. to 61 per cent.; Abies from 77 per cent. to 59 per cent. But that such is not constant appears from the comparatively low water-soluble pentosan content of Abies in the early months, and from the marked rise in the similar content in Cupressus from October to January, the average water contents for the three periods for which readings were taken being 57·0 per cent., 57·6 per cent., and 58·3 per cent.—variations easily within the ordinary environmental variations in any form. Further, the 1 per cent. HCl fraction exhibits an agreement with the statement of Spoehr on the Cacti, who claims a pentosan increase with decreasing moisture and increasing temperature. *Pinus montana* is the only exception where spring records are available. Of course, Spoehr's 1 per cent. HCl fraction really corresponds to our loosely combined total, which includes the water-soluble pentosans and those subsequently extractable with 1 per cent. HCl. In the plotted graph of our loosely combined total an agreement with Spoehr is not so obvious, but the comparisons just cited seem to indicate some connection between the water relationships and the pentosan content, a connection which must be further studied. We see, too, that though we can trace no connection

between frost-resistance and pentosan content, we meet again the old familiar fact that young plant organs with high water content are less resistant than older and more mature ones. In fact, as far as the frosts we ever experience here are concerned, the only danger period is this period of high water content. Again, an examination of the variations in the residue pentosan, only extractable by 12 per cent. HCl, and, till we know more about it, presumably in intimate association with the wall, shows that even such a pentosan type can vary in content throughout the year and differently in different forms. Pseudotsuga, Abies, and Tsuga, especially the latter two, agree in showing an increase from spring to summer, followed by an autumnal decrease in preparation for the winter, while in the Austrian Pine and the Sitka Spruce there is a minimum in late autumn, with a rise subsequently. *Pinus montana* is a law to itself. But in spite of the variations in the different fractions, the graphs of the total pentosan seem to show that all three are closely connected in the metabolism. Even the apparently aberrant forms Cupressus and *Pinus montana* fall into line, and permit of definite grouping with the Austrian Pine and the Sitka Spruce, where October shows a minimum content, with a marked rise from October to November. This group stands in contrast to the Abies, Pseudotsuga, and Tsuga group with a fairly constant value throughout the year till October, when a drop till November prepares for a winter constant. It is a great pity that spring and early summer readings of Cupressus were not available. If possible, that defect will be remedied this year.

Until much fuller figures are available, it is useless to interpret further the above estimations. We had no idea, at the start, of tackling the vexed question of the origin and fate of pentosans in the plant. We really wished by a comparison of the spring, summer, and winter pentosan content of Conifer leaves to seek evidence for, or against, Rosa's theory of hardiness. We are forced to admit that our figures for Conifers show no relation between such and pentosan content, and we now feel that, if such relationship is further substantiated in the cabbage and similar plants, it indicates either that the pentosan increase is in relation to the changed water conditions of the plants experimented with (a conclusion which can quite easily be drawn from Rosa's figures), or that plants like the Conifers have solved the problem of cold-resistance along other lines. We have not sufficient data yet to go further. But, before leaving these estimations of the pentosan content, we wish to point out that in the two classes in which greatest variation is evident—the water-soluble and the 1 per cent. HCl fractions—some degree of reciprocal relation exists. This is particularly clear in those cases in which greatest change takes place in the water-soluble fraction. Cupressus, *Pinus montana,* Pseudotsuga, and the Austrian Pine show the fact clearly that, during the period of greatest change in the water-soluble fraction, the total of loosely combined pentosan remains fairly steady. *Pinus montana* shows a break between August and October, but the rise from July to August in the water-soluble fraction is accompanied by very little change in the T.L. amount; while the T.L. records for August and November are very similar, although a heavy drop has taken place during the same period in the water-soluble total. The exact nature of this relation is not yet clear, but we propose to determine as soon as we can the nature of the pentosans characteristic of the three groups.

Hydrogen Ion Concentration.

Since it is well known that the power of imbibition of colloids can be markedly affected by the reaction of the medium, it seemed natural to test in some qualitative way at any rate the seasonal acidity. Rosa was able to show a

doubtful increase in titratable acidity in hardened plants, but could detect no variation in H-ion concentration. Only a few samples, however, were examined. There also exist other records of seasonal variation of acidity, from which it is still hard to determine either the result or the cause of such changes. Thus Hooker (9), working on the seasonal changes in the chemical composition of apple spurs, reports definite variation in the acidity, but only in the titratable acidity. H-ion concentration calculated as *pH*, however, remains singularly constant. Thus the bearing spurs of a variety called Wealthy showed a change in titratable acidity—given in c.c. of N/10 acid per gram of dry weight—from 2·04 in February to 3·40 in June, dropping to 1·74 in September. The corresponding *pH* figures are 5·8, 5·7, and 5·9. Although the *pH* is fairly constant, the titratable acidity increases from spring to early summer, drops to a minimum in autumn, and rises again in winter. On the other hand, Abbott (1) reports marked seasonal changes in the H-ion concentration of both apple and peach, claiming that the concentration is high in summer and low in winter. Apart from its effect on colloidal imbibition, we had the further interest in the *pH* values throughout the year, as it was just possible that the changes in starch and sugar content might be related to the H-ion concentration. Table 3 shows the result of our first survey of the ground. The estimations were carried out by a method which is not, perhaps, the most accurate one available; but, as far as they go, they do not indicate any important seasonal variation in the *pH* values. The method used was that previously used by Atkins (2), the leaves being crushed on white porcelain with one or two drops of distilled water, and the colours given by the suitable indicators compared against those given with standard buffer solutions. This may not be very exact, but it was at least exact to a difference of 0·5 in the *pH* value.

TABLE 3.

SEASONAL VALUES OF H-ION CONCENTRATION OF CONIFER LEAVES RECORDED AS *pH*.

	May 11, 1925.	June 12, 1925.	July 31, 1925.	Aug. 24, 1925.	Sept. 25, 1925.	Oct. 22, 1925.	Nov. 28, 1925.	Jan. 13, 1926.
Austrian Pine ...	3·8	3·7	3·9	3·5	3·7	3·6	3·6	3·9
Pinus montana ...	—	—	—	3·6	—	3·8	—	—
Tsuga	3·5	3·2	3·3	3·5	3·5	3·5	3·6	3·5
Abies	3·7	3·5	3·7	3·5	3·8	3·7	4·3	3·7
Sitka Spruce ...	3·5	3·4	3·5	3·7	3·7	3·7	3·7	3·7
Cupressus ...	—	> 4·6	> 4·6	5·1	5·1	5·0	4·8	5·1
Juniperus ...	—	> 4·6	> 4·6	5·2	5·3	5·4	5·3	5·3
Larch (Eur.) ...	3·3	3·5	3·8	4·1	3·8	4·3	Fallen	Fallen
Douglas Fir ...	—	—	—	3·7	—	—	—	—

The indicators used were Brom-phenol Blue and Di-ethyl Red, with a range respectively of 2·8—4·6 and 4·8—5·4. Methyl Red 4·4—6, Methyl Orange 2·9—4, and Dimethyl amino-azo-benzene 3—4, were also used, the latter, however, being quite unsatisfactory, the other two acting very usefully as checks. We feel confident that these figures are approximately correct. Each determination was made many times, and the services of anyone available, on whose judgment any reliance could be placed, were commandeered to match colours, he being without any bias towards one point or another. Further, the column under May 11th was checked against standard buffer solutions, varying 0·2, made up by ourselves from the B.D.H. Universal solution. Fearing that our own solutions might be faulty, we obtained guaranteed solutions from B.D.H. in a wide series, differing by 0·2,

each of which we tested colorimetrically as carefully as possible. The new solutions, however, gave us readings in June closely approximating to those we had already obtained. The November value of *Abies pectinata*, 4·3, is apparently aberrant, but we feel quite confident of it, though unable to explain it. The only form in which there appears any appreciable regular change is in the Larch, which decreases in acidity with advance of the year. Unfortunately, more by an oversight than anything else, *Pinus montana* and the Douglas Fir were neglected, but, luckily, just those two months in which the greatest change took place in *P. montana* in the W.S. pentosan fraction have the corresponding *pH* recorded, and show values which are in close agreement. Two types, however, stand out with much higher *pH* values than the others, viz., *Cupressus Lawsoniana* and *Juniperus communis*. For the June and July readings no Di-ethyl Red was available; hence we did not decide on the exact value without some check. The readings were outside the range of Brom-phenol Blue, and Methyl Red, which was not sufficiently satisfactory to decide on by itself, gave readings about 5·1 for Cupressus and 5·3 for Juniper; but we have simply entered it in the table as higher than 4·6. This departure in Cupressus is of interest, since Cupressus is the form whose W.S. pentosan fraction is unique. Now, though Juniperus has not been yet dealt with from the pentosan point of view, it has a double interest. In the first place, its closer relationship to Cupressus may be significant. It seems necessary to extend our observations on the H-ion concentration and the pentosan content of other species of these genera and of other members of the Cupressineae. But it has a greater interest, which we hope to deal with in a further communication, inasmuch as the capability of starch formation by the Juniper in winter is greatly in excess of other forms with which we are at present experimenting, such as the Austrian Pine and the Hemlock. But, whatever other interest may subsequently attach to this question, we can safely say that there exists no obvious relation between the seasonal pentosan variations and the H-ion concentration.

Conclusion.

It seems, therefore, as far as Conifers are concerned, that there is no relationship between the pentosan content and the relative hardiness of different types, nor between the pentosan content and seasonal hardiness. If we limit ourselves to the water-soluble fraction emphasized by Rosa, we find the minimum, and a very low minimum, during the period of greatest hardiness, and certain important species, e.g., the Douglas Fir, the Silver Fir, and the Sitka Spruce, which are quite sensitive to spring frosts, and really only frost-sensitive then, have their maximum content during the sensitive season. Further, it seems to be assumed by Rosa that pentosans and similar colloids really do hold water against the powers of dehydration and ice formation. He produces, as further support of the theory, the fact that hardened leaves with a greater pentosan content dry more slowly than non-hardened leaves. With this aspect of the question we hope to deal briefly in a second communication, but we are in no way satisfied that even the physical basis of the theory is correct. It would have proved more satisfactory if Rosa had shown carefully, as a preliminary, that pentosans do act as they are claimed to act. Again, in his hardened plants he finds, not only a pentosan increase, but an increase in the sugar content. His work is, therefore, as much a support for Lidforss' sugar theory as for his own pentosan theory. Moreover, as we find in other forms no relation between hardiness and pentosan content, but seem always to find an increase of sugar in the hardened, frost-resistant, and winter-green plants, his results seem actually to be further proof that the key to the problem

of frost-resistance lies in the sugar increase. This, however, is not yet the place to deal with the whole question. Nor can anything be added to theories concerning the functions of pentosans. Until we have made sure of the chemical nature of the three classes, for instance, and can, possibly, place them in the plant cell, speculation seems useless. But if pentosan content can vary, as it does, throughout the year, it seems obvious that some enzyme group must be responsible for the changes, and that the metabolism of the pentose sugars may be of greater moment in every group of plants than it has so far been considered to be.

SUMMARY.

1. Attention is called to the lack of knowledge available concerning the physiology of evergreens and particularly of Conifers.

2. The theory that cold-resistance depends on pentosan content has been examined by a seasonal study of Conifer leaves.

3. The Pentosans of Conifers can be classed in three groups: (*a*) water-soluble pentosans, (*b*) pentosans subsequently extractable with 1 per cent. HCl, and (*c*) pentosans still remaining, and only extractable with 12 per cent. HCl.

4. All three classes vary considerably throughout the year, though not independently, the variation in classes (*a*) and (*b*) being reciprocal.

5. Cupressus, of the forms examined, is remarkable in having a very high winter content of water-soluble pentosan, this class being usually present in minimum quantity during the winter months.

6. The H-ion concentration of Conifer leaves seems to show little seasonal variation. The leaves are distinctly acid — from *pH* 3 to 4. Cupressus is distinctly less acid than the other forms examined.

7. Analysis of the full results shows that in Conifers no relations, seasonal or otherwise, can be established between hardiness and pentosan content.

8 The theory is considered unnecessary and unsupported.

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No. 22.

THE DOWNY MILDEW OF ONIONS (*PERONOSPORA SCHLEIDENI*), WITH PARTICULAR REFERENCE TO THE HIBERNATION OF THE PARASITE.

By PAUL A. MURPHY, Sc.D., M.R.I.A.,

AND

ROBERT M'KAY, A.R.C.Sc.I.

[Plates XII–XV.]

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In a note published in 1921 (12) attention was drawn for the first time to the fact that the mycelium of the downy or false mildew fungus of the onion, *Peronospora Schleideni*, Unger, was capable of penetrating the scale-leaves of onion bulbs and of hibernating there. The observation then made has been amply verified since, and the present paper deals more fully with the work which has been carried out on this subject as opportunity presented itself during the last five years.

Attention may be drawn again to the suitability of infected onions for demonstrating intercellular mycelium and haustoria, for both are so large that they can be seen even in rough preparations and without staining, while material is easily found at all seasons of the year.

Finding of Perennial Mycelium in Onion Bulbs, and its Connection with Peronospora Schleideni.

The observations made in 1920 and 1921 were as follows:—Non-septate mycelium, provided with characteristic long filamentous haustoria, which had the appearance of that of a higher Oomycete, was found abundantly between the cells in the scale-leaves of potato- and ordinary onions and shallots in May and June, 1920. It permeated all the scale-leaves, as a rule, from the outer ones, which were brown and rather watery, to the still crisp and white inner ones, and was present also in the rudimentary central shoots. In the case of infected potato-onions which had already divided into a number of smaller bulbs, the latter were also found to be affected throughout. Four lots of onions or shallots of different origins were found diseased in this way.

Attempts to induce the fungus to fructify on the scales were unsuccessful, no mycelium growing out whether the material was kept in moist air or in water, or on the surface of oat-agar. An attempt to infect healthy bulbs directly was similarly unsuccessful. In one case the mycelium was traced directly, and without an apparent break, from a scale to the green upper portion of the same leaf, and conidiophores of *Peronospora Schleideni*, which

were present on the surface, appeared to spring from this mycelium. It was therefore considered probable that the mycelium in the bulbs belonged to this fungus, the only possible known alternative being *Phytophthora allii*, Sawada (14), with which it did not appear to agree so well, and which, moreover, does not appear to attack the common onion.[1]

Later in that year further infected bulbs were found, from which it was learned that the same mycelium invaded the stem or root-stock, and here again some of the green shoots—but not all—contained this mycelium; but an attempt to induce the fungus to fructify on the leaves in a greenhouse during the winter ended in failure, the bulbs being too seriously affected to permit of normal growth. It was not until the following spring that the connection between this mycelium and *Peronospora Schleideni* was definitely established.

In May, 1921, the following plants, which had all passed the winter together on the roof of the College of Science, in the centre of Dublin, were available for study:—Nineteen plants which had been under observation in 1920 and were then free from mildew, and four plants which had been mildewed in 1920. On examination, one of the latter was found to contain non-septate mycelium in the green shoot, but the other three, which were in a separate pot, contained none. There was no external sign of mildew or other disease on any. Both pots were covered with bell-jars on the evening of May 6th, and on the following morning the single plant in which mycelium had been proved to be present was covered practically all over from the base up to and including the flower bud with conidiophores bearing conidia of *P. Schleideni* (Pl. XIV, fig. 25). There was no mildew on the three plants which were free from mycelium, and none appeared afterwards, although they were kept covered for twelve days. The diseased plant was dug up and examined the day the mildew appeared, and was found to be permeated with non-septate mycelium from the basal end of the root-stock to the extreme tip of the flower bud, and from this mycelium conidiophores of *P. Schleideni* were being produced. The three other plants were dug up after being covered for twelve days, when no mildew was present, and each was found free from mycelium in all its organs.

Furthermore, the other nineteen plants which had been healthy in 1920 were placed under bell-jars for periods of five to seven days, or were dug up and placed in moist glass dishes for the same period, and they also failed to develop mildew, and were all found on individual examination to be free from internal mycelium in bulbs, leaves, and shoots.

The behaviour of these control plants proves that there was no external infection, and the development of mildew on the only plant in which the mycelium hibernated shows that this mycelium belongs to the onion mildew fungus.

Further proof of this was secured about the same time. Shallots which had been planted in January and February in the open were discovered in the first week in May to contain abundant non-septate mycelium, although there was not much external sign of disease. There was a trace of mildew on some of them, but none was present on most. When, however, they were placed in a moist atmosphere for one night, an extraordinary and uniform growth of mildew developed all over most of the leaves, except near the tips, where these were even slightly yellowish, and for about one-sixth of the length near the base (Pl. XV, fig. 27). The fact that the disease was found to break out so early

[1] Thanks are due to Dr. E. J. Butler, Imperial Bureau of Mycology, for supplying Sawada's paper and a translation.

in the year and so generally over the plant, and that it was associated with a mycelium which permeated the whole bulb, points clearly to systemic infection originating in a previous season.

That this was indeed the fact was proved in the case of another lot of shallots which had not been planted. Two of these, which had sprouts about 1 inch long, were found to contain the familiar non-septate mycelium throughout in bulbs and shoots, but there was no mildew on the surface. These shoots were cut away, and the bulbs were planted in pots filled with sterilized soil. Another similar pot was planted with bulbs of the same lot which were proved to be free from mycelium. One of the diseased bulbs produced new shoots, which, as they came up, were again found to be permeated with non-septate mycelium. Nineteen days after planting, both pots were covered with bell-jars, with the result that the only surviving plant from the bulbs containing mycelium became practically covered with mildew within twenty-four hours (Pl. XIV, fig. 23), while the control plants showed none after two days, and were then, on examination, proved to be free from mycelium throughout.

The cumulative evidence obtained in this way indicates unmistakably that the non-septate mycelium which was found at all seasons of the year in onion bulbs was that of *P. Schleideni*, and that, therefore, this fungus can hibernate in the vegetative condition in these organs, with the result that the leaves and shoots produced in the following season are generally infected from the beginning. As stated in the previous note (*loc. cit.*), there is a time in the spring when the only infections found are systemic ones, derived from mycelium in the bulb, a fact which can be verified by tracing the distribution of the mycelium, and which can even be told from the outside by the place in which fructifications appear, and by their extent. (See section on Development of Shoots from Diseased Bulbs, p. 242.)

Further Proof of the Connection between the Mycelium in the Bulbs and Peronospora Schleideni.

Following a very severe outbreak of mildew in the year 1922, a very large number of the onion bulbs which grew in a plot at Glasnevin were found in the autumn of that year to contain the mycelium of *Peronospora Schleideni*. Most of the infected bulbs sprouted prematurely, and the same mycelium was found freely in the green shoots; but numerous attempts, some of which were continued for periods of over one month, to induce the fungus to fructify on the shoots of bulbs which had not been planted met with failure.[1]

On December 15th, 1922, four of these bulbs belonging to the variety Wethersfield Red, which contained very abundant mycelium, were planted in a large pot, which was kept in an unheated greenhouse. Two of them produced plants, which were fairly vigorous and normal in appearance, although of an unusually dark green colour. On March 8th the plants were covered with a bell-jar, and seven days later mildew appeared abundantly on one of the plants (the more vigorous), and sparsely on the other, which was obviously ailing, although it was permeated with mycelium. The mildew appeared in the manner which is characteristic of systemic infections, namely, first on the upper portion of the older leaves (cf. Pl. XIV, fig. 24). The maximum temperature of the house when conidiophores were first produced was 50° F., but a few days

[1] An individual case was met with in November, 1925, of the mildew fungus producing conidiophores and conidia sparingly on the green shoot of an infected bulb in storage, thus furnishing further proof of the identity of the fungus.

later, when it rose to 74° F., they became much more abundant. There were no other onions in or near the house, nor was it possible, in view of the very dry and cold weather, for air-borne infection to have occurred at that time.

A further lot of bulbs which were proved to contain the same mycelium were planted in the open air on March 9th, 1923, in a plot which had grown no onions for at least fifteen years, and which was separated a considerable distance from any other onions. At the same time bulbs from the same lot which were proved to be free from mycelium were planted in another similar isolated spot. The results were as follows:—

Non-septate mycelium in bulb.	Variety.	No. of bulbs which— were planted.	survived.	developed mildew before end of May.
Present ...	Up-to-Date ...	31	14	4
do. ...	Wethersfield Red ...	19	7	7
Absent ...	Up-to-Date ...	10	10	0

In addition to the ten healthy Up-to-Date bulbs mentioned, eight proved healthy potato-onions, and three healthy shallots were planted in the same place, and they also remained healthy. No bulbs of Wethersfield Red could be found which were free from non-septate mycelium.

As may be seen from the table, more than half of the affected bulbs, which were generally very badly diseased, succumbed; and, of those that survived, four out of fourteen of the Up-to-Date plants and all the plants of Wethersfield Red developed mildew during May. It may be noted that the former is resistant and the latter very susceptible to mildew. As indicated by the behaviour of the controls, which all remained healthy, infection is again to be traced to the mycelium which was present in the bulbs.

It will be seen in a later section (p. 244) that plants were infected by spraying the foliage with conidia of *Peronospora Schleideni*, whereby mildew was produced on the surface, that the bulbs became infected from the foliage, and that in turn the same non-septate mycelium invaded all the shoots which were subsequently produced, with the result that mildew developed on them in the following March. As control plants remained free throughout both from internal mycelium and from mildew, final proof is thus furnished of the identity of the non-septate mycelium.

The Disease in the Bulbs.

Diseased bulbs produce green shoots prematurely (Pl. XIV, fig. 26), as early as the end of the old year or early in the new, and turn rather soft, until, in bad cases, there is no more solid substance left. Such extreme examples generally produce quite large leaves, but very few roots, and are commonest in the most susceptible varieties, such as All-the-Year-Round, Stirling Exhibition, and Wethersfield Red, which may become a total loss by early December.

Less severely attacked bulbs, mainly of the variety Up-to-Date, frequently do not sprout until about February, the flesh remaining firmer both before and after sprouting than in the previous cases. One of the first indications of a diseased condition is that the dead outer scale, and often the first living one, are somewhat corrugated and rough in places. In advanced stages the presence of disease may be inferred from the shrivelled appearance and soft feel, but in

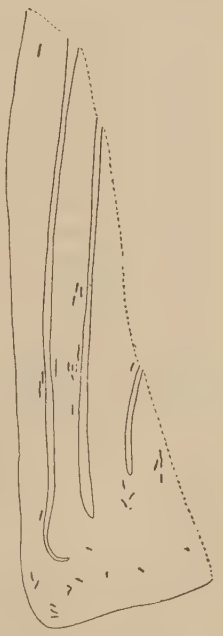
many cases it can be established with certainty only when the dry outer scales are removed. The first fleshy scale is then seen to be corrugated, shiny, brownish or amber-coloured, and watery in appearance, or to have areas of this nature, with very indefinite margins, which may occur on any part of the bulb, top, bottom, or sides. The discolouration is confined to the outermost still living scale leaf, for the scales inside are found on cutting to be pure white, fleshy, and crisp, and to all appearance healthy.

The discoloured areas have no connection with the distribution of the mycelium, for in the inner healthy-looking scales, as also throughout the outer discoloured one, there occur very abundantly between the cells much-branched non-septate hyphae of large but irregular diameter, which are hyaline except in scales which have turned brown, where they also may take on this colour (Pl. XII, fig. 5). Swellings of a round, oval, or irregular shape, either intercalary or terminal, are frequent on the mycelium. These are not cut off by septa, and are filled at first with fine protoplasm, without any trace of differentiation at any time. The contents are similar to those of the hyphae, but in old mycelium the protoplasm becomes granular, and may not fill the cavities, as may be seen in the outer scales, for the vigour of the fungus increases towards the centre of the bulb.

The mycelium is freely provided throughout with characteristic haustoria, which penetrate the cells. These are convoluted simple tubes of comparatively large size, which are of approximately the same diameter throughout, except that they are somewhat narrower where they pierce the cell wall. In this part of the plant they are generally unbranched, but branched specimens also occur. The cells are entered through a simple opening in the wall, and no collar or other swelling develops around the invading haustorium.

The effect of the mycelium on the bulb is at first strikingly little, even where there are abundant haustoria. Cells penetrated by the latter are not killed immediately, or even for a very long time; the tissues are not broken down, and there is no obvious loss of turgor. In sections of invaded scale leaves, however, the cytoplasm and nuclei of the host are not so obvious as in healthy tissue.

The compressed stem or root-stock is also invaded throughout (Pl. XII, figs. 1 and 2). The mycelium may be found in the meristem and deeper layers in microtome sections, but it occurs in greater quantity near the upper surface. Minute haustoria are also present. The rather finer and much convoluted hyphae run irregularly between the small and closely packed cells, and from there make their way into the new shoots (Pl. XII, fig. 3). A direct connection has been traced between the mycelium here and that in the bases of the leaves. The roots are not attacked.



TEXT FIG. 1.

Section through portion of bulb of young autumn-sown onion, showing position of mycelium in scale-leaves and stem. (× 12.)

Bulbs when examined in winter are found, as a rule, to be invaded throughout, but there are exceptions. Similarly most of the green shoots (flowering and leafy) produced by such bulbs are infected from the beginning (Pl. XII, figs. 6

and 7), as evidenced by the distribution of the mycelium from base to tip (where, however, there is no particular concentration of it), and by its presence among the cells of young leaves only a few millimetres long, which are still contained within the bulb. Cases were also found, however, in which leaves were being gradually infected from the base after they had grown a considerable length, while, exceptionally, some or all of the shoots may escape infection. Once the shoots are invaded they remain infected throughout, for the parasite keeps pace with their growth, and does not die off or become less vigorous in the basal portions.

Development of Shoots from Diseased Bulbs.

The mycelium is found to be extremely vigorous in young leaves developing from affected bulbs. In these young shoots it occurs characteristically in long straight strands (Pl. XII, fig. 4), which may sometimes be traced for several centimetres running vertically between the elongated cells of the leaf, either just within the main chlorophyll-containing layer of cells (which is two to four cells deep), or near the inner margin, particularly in the neighbourhood of a vascular bundle (Pl. XII, figs. 8 and 9). Haustoria are present throughout, and the swellings on the hyphae already described are sometimes met with. Afterwards, when the bulb has been planted, the parasite penetrates between all the cells, and it is only when this stage is reached that fructification becomes possible.

The conditions necessary for the production of conidia include a vigorously growing plant and a certain age of leaf, or more probably a degree of maturity of the mycelium within it. Fructifications do not appear at all, or, if they do, only in minute traces, on very weak plants; and the same holds for portions of plants detached before the fungus is ripe.

Before the stage of fructification is reached a relatively enormous network of much branched and convoluted hyphae of large diameter develops in the more specialized assimilating parenchyma (Pl. XIII, figs. 11 and 12). Finger-like processes are thrust in between practically all the cells, and branches penetrate as far as the inner wall of the epidermis, while inside the stomata many hyphae are to be seen ready to emerge and form conidiophores under suitable conditions. The hyphae are densely filled with uniform non-vacuolate protoplasm, and they are abundantly provided with haustoria, two or three of which are sometimes seen in the same cell. The haustoria are larger and more branched than the majority of those in other tissues, such as bulb scales and root-stock, and they also contain more abundant protoplasm, which, however, is more vacuolate and less dense than that in the adjacent mycelium (Pl. XII, fig. 10). Leaves containing the fungus in this stage produce conidia within twenty-four hours, or less, under moist conditions, whether detached from the plant or not.

That a certain stage of development of mycelium within the leaf is a pre-requisite for the formation of conidia is shown by the failure of conidiophores to appear on the young leaves of systemically infected plants containing abundant mycelium, when the older leaves are covered with mildew (cf. Pl. XV, fig. 27). The conidiophores first break out on the more mature leaves, and generally on the upper portions of these, and this, in fact, is diagnostic for systemic infection, coupled with a general distribution of the mycelium inside.

The production of conidia on the terminal portion of the leaf of a systemically infected plant is shown clearly in Pl. XIV, fig. 24.

External evidence of the presence of the parasite becomes visible after the general invasion of the chlorophyll-containing tissues, as a yellowing of the leaves. This arises from the death of individual cells which have been invaded by haustoria. The chlorophyll grains first become irregularly distributed or aggregated in groups (Pl. XIII, fig. 13), and then lose their identity, resulting in a uniform green slime. A yellowish colour then appears, and the green finally vanishes. As the cell begins to die, the contents of the haustorium become coarsely granular, and largely disappear.

Conidiophores and Conidia.

Conidiophores arise from the stomata[1] as thick-walled, straight, unbranched tubes, stout below and becoming narrower above. As a rule they grow at right angles to the surface; but when a leaf is bent outwards at a considerable angle, they are more abundant on the upper and inner surface (Pl. XIV, fig. 24). The same feature is sometimes even more marked in the case of detached leaves laid horizontally on supports in a dish, where, although free to grow out in all directions, conidiophores are produced mainly on the upper surface, while those produced at the sides bend sharply, apparently in the direction of the source of light. The stalk later branches four or more times, the laterals branch about three times, and the sub-laterals again once or twice, each of the last members producing two of the peg-like protuberances on which the conidia are borne. The growth in length after each succeeding division is shorter, until the branches finally end in short tapering projections, which are curved (Pl. XIII, fig. 16). Septa have never been seen in the main stalk of the conidiophore, but they do sometimes occur in the branches at the points where they bifurcate. More than sixty conidia may be produced on as many terminals of one conidiophore. The conidia are never produced successively from the same point. The length of the conidiophores varies from about 500 to more than 900μ, and the diameter at the base from 12 to 15μ.

The conidia first arise as spherical swellings on the tips of the ultimate branches, which then cease growth (Pl. XIII, fig. 14). As they increase in size, the conidia become obovoid in shape, being attached by the narrower end (Pl. XIII, fig. 15), and at maturity they drop very easily, and are seldom found attached to the stalk. The mature conidia are about twice as long as they are broad, with flat sides, which taper up to one end, where they are cut off square (the point of attachment), the other end being rounder, with a blunt point in the middle (Pl. XIII, fig. 17). The average dimensions of thirty-five conidia were found to be 52·5 × 27μ, the limits for length being 69 and 43·5μ, and for width, which is more constant, 30 and 22·5μ.

Conidia when freshly produced are almost, but not quite, hyaline, and the effect produced by them in the mass is of a grey coating on the leaves. There is a faint colour of brownish purple in the wall, which is not easy to define,

[1] In several cases conidiophores and conidia have been found within the leaf. These arise from the failure of a conidiophore to find a stoma through which to emerge, with the result that it runs up to the inner wall of the epidermis, turns sharply at right angles, then, as a rule, turns inwards again (evidently on meeting the side wall of a palisade cell), and branches and forms distorted conidia among the cells of the spongy parenchyma.

but approaches most nearly to Ridgway's "ecru-drab." This becomes accentuated with age, and leads to the usual description of the mildew as presenting a purple appearance.

Germination takes place by the production of one or more germ tubes from any part of the conidium (Pl. XIII, figs. 18 and 19), but approximately one-half of them germinate at the narrower end. The life of the conidia is evidently very short, as other investigators have found (16, 11, 23), for it is sometimes difficult to procure viable ones. Tap water or soil water is the best medium for germination, onion extract practically inhibits it altogether, but germination has been found to take place on the surface of oat-agar. Oxygen is also very necessary, as may be seen in cover-glass preparations, where only conidia near the edge germinate. In moderately deep layers of water which are exposed to the air the conidia produce germ-tubes which are uniformly twisted at first (Pl. XIII, fig. 20), but those on the surface germinate better than those which are submerged. Branches, some of which spring from appressorium-like swellings, and are of more limited growth, while others resemble the initial stages of a mycelium, are produced by germ-tubes which develop in tap water; but it is in soil extract that this development has been observed to go farthest (Pl. XIII, fig. 21), which probably indicates that the conidia or their products will survive for some time in the soil.

Even when germination experiments are carried out with conidia which have been produced over night under bell-jars, with every precaution observed to guard against desiccation, it is extremely difficult to procure uniform material. From our experiments in this connection it may be concluded, however, that germination is possible at the lowest temperature used, namely, 8·5° to 10° C., while a trace was found at 25° C., but none at 30° C. It is evident that germination can take place over a wide range, but the exact optimum was not determined. Continued darkness appeared in two experiments to promote germination.

Progress of the Disease from Leaves to Bulbs.

Local infections on the leaves are marked by the development of an easily overlooked pale green or yellowish colour around the point of infection, which shades off imperceptibly into the normal green. Infection may occur on any part of the leaf, but it appears to be commonest about the spot where one leaf emerges from the other, due, no doubt, to the retention of conidia and water there. Once infection has taken place, the mycelium spreads rapidly within the host, apparently more so in a downward than upward direction, and infection of the bulb follows rapidly, as will now be seen.

Onions of the variety Ailsa Craig, which were raised from seed in a pot in a greenhouse, were inoculated while they were still seedlings by spraying the leaves with a suspension of the conidia in water, the germination of which was found to be moderate. The plants in another similar control pot were sprayed with plain water, and both were covered for two days, and were then kept in the greenhouse. In fourteen days yellowish areas appeared on the leaves of some of the inoculated plants, and these were found to contain the familiar mycelium, which was proved to be that of *Peronospora Schleideni* when conidiophores were found on the twenty-third day. It was with considerable surprise that the mycelium was then discovered to have already reached the bulb, and actually to have spread from the stem into the young central leaves. The dis-

tribution of the parasite twenty-three days after infection is shown in tabular form in the case of three plants, and is illustrated graphically in text fig. 2.

Plant.	1st leaf (oldest).	2nd leaf.	3rd leaf.	4th leaf.	Stem
1	+ from tip of leaf to stem. This was leaf originally infected.	+ in scale and neck up to 5·5 cm. from base. – above.	+ in scale and neck up to 7·5 cm. from base. – above.	+ from stem to tip of leaf (leaf 4·5 cm. long).	+
2	+ in isolated spot half-way up. – below.	– throughout.	– throughout.	– throughout.	–
3	+ from about point of infection (? tip) to stem. This was leaf originally infected.	+ in scale and neck up to 3 cm. from base. – above.	+ in scale and neck up to 5 cm. from base. – above.	(No leaf).	+

Many of the plants which were sprayed with the spore suspension escaped infection in their leaves, and these were invariably found free from invasion throughout. Similarly, many plants in the control pot were examined, and proved to be entirely free from mycelium in all parts, and no plant here ever developed mildew. The course followed by the parasite in its development is, therefore, clear. All three infected plants were attacked first in some part of the green portion of the oldest leaf, and in two cases the fungus had made its way down through the base of this leaf into the stem (or root-stock), and from there was growing up into the remaining leaves. These were being invaded whether they were already of considerable length when the parasite reached their bases (as in the case of the second leaves), or were quite small, and the smaller they were the further the fungus had advanced in them, until eventually they became diseased to the tip. (See text fig. 2.)

In an earlier stage of this work it was believed that there was some evidence that the mycelium might spread direct from scale to scale within the bulb. Later observations have not confirmed this, and the fact, which was soon afterwards discovered and amply confirmed since, that the stem is also invaded, and that a connection can be traced between the mycelium there and that in the base of the leaf (Pl. XII, fig. 3), makes such an explanation unnecessary. Furthermore, our observations are most readily explained by assuming that the method of spread in the bulb is through the stem alone, as sometimes an outer infected scale is found to be separated by one or more non-infected scales from the young central leaves, in which mycelium is present.

The few remaining plants in the pot which was sprayed with conidia in this experiment, and their controls, were kept in a greenhouse until March of the following year, when they were covered with bell-jars, which remained in position for five days. Within two days conidiophores of *Peronospora Schleideni* broke out on the leaf of one infected plant from the tip to about three-quarters way down. This plant was found to be permeated with mildew mycelium from stem to leaf-tip, and incidentally some resting-spores were found in a localised area on its oldest leaf, which was dead. The plant was yellower and less vigorous

than the others. No other plant in this pot developed mildew or contained mycelium, and no plant in the control pot did either.

TEXT FIG. 2.

Diagram illustrating distribution of *Peronospora* in seedling onion twenty-three days after infection, the shaded portion being that invaded by the parasite.

Attempts to Infect Onion Bulbs Directly.

Previous attempts, already referred to, having failed to infect bulbs by inserting in them portions of diseased leaf-scales, conidia from mildewed leaves were used for the same purpose. Healthy bulbs, which were stripped until a

living scale was exposed, and were lightly disinfected on the surface, were laid in watch glasses containing conidia in water, while control bulbs were similarly placed in plain water. Under these conditions the germination of the conidia was poor, although good on control slides; and the experiment was repeated, therefore, with conidia which had already germinated in water. In another experiment water containing germinated conidia was poured into incisions in onion bulbs. All these bulbs were incubated for more than five weeks, but careful examination showed that no infection had taken place.

A further effort was made to infect young onions (Ailsa Craig) growing in soil in a pot by means of conidia applied around the bulbs. The onions were raised from seed in fresh soil from a pasture field, and before the seed was sown a large cylindrical glass chimney was fixed upright, with one end just beneath the surface of the soil, and closed at the other end with cotton-wool, so as to exclude aerial infection. A bent glass tube ran under the edge of the chimney, and terminated just below the surface of the soil in the centre. When the onions were about three inches high, a suspension of conidia in water was introduced through the glass tube, just sufficient being used to make the soil look moist, without any free liquid rising above the surface. A control pot was similarly treated, but plain water only was added. The first lot of conidia did not germinate very well, and eight days later the process was repeated with conidia which germinated pretty well. The pots were under observation for about eight weeks, but no infection resulted, as was proved by observation and microscopical examination.

Several other experiments were carried out in 1921 and 1923, in part with soil which might be presumed to be naturally infected, owing to its having carried a badly diseased crop, and in part with soil contaminated by digging into it bulbs and leaves containing mycelium of the mildew fungus. Control pots were provided in all cases, and onion seed was sown in them and in the pots of contaminated soil; but no mildew resulted in any case, nor did any of the plants contain mycelium of the mildew fungus. The same result followed from an experiment carried out in the open with presumably contaminated soil *in situ*. It is not necessary to give details of these experiments, which were carefully carried out, and were kept under observation for periods of three to six months.

In view of these uniformly negative attempts to infect the bulbs directly, and the proved ability of the fungus to penetrate the bulbs from the leaves, it is safe to conclude that the latter is the only method by which the bulbs are invaded under natural conditions. There is little or no possibility of the disease spreading in storage, although it may sometimes happen that the fungus produces conidia on the surface of green shoots from diseased bulbs in very damp weather in autumn or early winter. If the disease is spread by these, it may be assumed that any infections which take place would be through the leaves.

Resting Spores and their Practical Significance.

The question now arises: to what extent does perennial mycelium in the bulb serve as a means of hibernation, and how far is it supplemented by resting spores or other bodies? Resting spores do occur, but apparently only rarely under our conditions at least, for in the large amount of infected bulbs and

foliage which has passed through our hands they have been found only twice, in old dead leaves. In these cases, however, they were abundant in local areas, and could not easily have been missed. They have never been seen in infected bulbs, although these have been examined in all stages on purpose. Our present opinion about them, which may, of course, have to be modified by further experience under a wider range of conditions, is that they play a secondary part in perpetuating the fungus. It is of interest to note in this connection that, apart from Smith (17), Shipley (16), Jones (10, 11), and Whetzel (23), the resting spores do not appear to have been seen by any investigator of the disease, while Thaxter (20) and Sorauer (18, 19) state that they did not observe them.

The sexual organs (Pl. XIII, fig. 22), which occur in the mesophyll, are colourless during early developmental stages, but later have a distinct yellowish appearance, and cannot easily be overlooked. The antheridium, which is paragynous, is irregular in shape, and is generally difficult to make out, owing to the mass of mycelium present, but it has been found to measure about 23 × 15μ. A fertilisation tube, the remains of which can sometimes be seen at later stages, runs from it into the oogonium. The oogonium is roughly spherical in shape, and measures about 35 to 45μ in diameter. The colour of the oogonium becomes yellow as development proceeds.

The oospore (Pl. XIII, fig. 22) is nearly spherical in shape, and, as may be seen when removed from the oogonium, it has a smooth wall, which reaches a thickness of 3 to 4·5μ in well-formed specimens, and is colourless. The diameter was found to vary from 28·5 to 35μ, the average being 31μ. The spores in one of the two lots of material were not so thick-walled, and presented a general appearance of being imperfectly formed or matured, while in the other, though far fewer in number, they appeared to be perfect. The statement which occurs throughout the literature that the resting spores are thin-walled is probably based on the examination of immature or imperfect specimens. The germination of the oospores has not yet been observed.

If the resting spores are not of commoner occurrence than our experience indicates, they can play only a minor part in perpetuating the fungus. It may be that this species of *Peronospora* has found, like so many others of this genus, that persistent mycelium in a perennial host is a surer means to the same end—in that the connection between host and parasite is not broken, and the chances of finding new hosts in the vicinity of the old are increased—and is, therefore, losing the faculty of producing resting spores.

A number of observations point to the fact that the production of conidia and of resting-spores are antagonistic processes, for, when the former takes place, the great reserves which the fungus had previously accumulated in the leaf are, as we have found, largely used up, with the probable result that resting-spore formation is prevented. On the other hand, attempts at the production of resting spores (for thus we interpret some of the imperfectly differentiated swellings shown in Pl. XIII, fig. 22, which resemble very closely the abortive oogonia of *Phytophthora infestans*) are most frequent in leaves which have not been in a position to produce conidia, and the best lot of resting spores we have seen were produced under similar conditions in a dry greenhouse. On this hypothesis the rarity of resting spores under Irish conditions, where conidia can be produced at most times throughout ten or eleven months of the year, is readily explained, while under other conditions they may be much more abundant.

Practical Significance of Perennial Mycelium in Ireland and its Probable Occurrence Elsewhere.

The frequency with which infected bulbs occur in Ireland certainly points to perennial mycelium being the chief method of hibernation. Some particulars regarding this have already been given, the most notable example being that, following a very severe outbreak of mildew in 1920, practically 66 per cent. of the smaller bulbs from one plot were found to be infected. Infected bulbs have been found readily every winter since whenever they were required, even following the dry season of 1925, which was unfavourable for mildew. In this respect the fungus is in at least as good a position as *Phytophthora infestans*—for infected bulbs are probably commoner than blighted potatoes—and is in a better position so far as the production of aerial conidia is concerned, for infected shoots are not, as a rule, killed off before fructifications can appear, as is so often the case with blight.

That infected bulbs are an important source of new infections in the spring, and have been so for long, is indicated by the observation of gardeners and the views of the earlier workers on this disease. After our first discovery of perennial mycelium in the bulbs, it transpired that Mr. Irving, Horticultural Inspector in the Department of Agriculture, had for long connected the first outbreaks of the disease with a certain type of soft bulb, some of which were supplied by him and found by us to contain mycelium of the mildew fungus. Similarly, according to Whetzel (23), the two earliest workers on the disease in America believed that the fungus hibernated in the bulbs. He says (p. 156):—"The earlier investigators believed that the fungus wintered as mycelium in the bulbs of onions, and produced conidia again when the host was planted the following year." And again: "Trelease (1884) and Dudley (1889) both believed that the mycelium passed the winter in bulbs of affected onions." This, however, was not proved, and Whetzel himself, not having found any mycelium in the bulbs of badly infected plants, doubts the extent to which the mycelium is perennial in the host, although not excluding its possible occurrence. Jones (10, 11), while similarly recording the views of some of the earlier workers, also failed to find mycelium in the bulbs.

The presence of mycelium of the mildew fungus in the bulbs is not recorded by any of the other investigators of the disease, among whom may be mentioned Berkeley (2), de Bary (7), Shipley (16), Thaxter (20), Eriksson (8), Sorauer (18, 19), and Ritzema Bos (13). The paper of Thaxter referred to is short, but very suggestive. He noticed in the early part of July, 1888, "that, in all situations, only seed onions [onions grown from bulbs for seed purposes] seemed to be thus injured; and, although in many cases seed onions were growing almost side by side with market onions of the same variety, the latter were not attacked in a single instance, so far as observed." The probable explanation is that the fungus had hibernated in the bulbs and then fructified on the shoots they produced, but had not yet visibly attacked the onions grown from seed. It is noteworthy that Thaxter failed to find resting spores in the tissues, although he believed that the parasite was dependent on them for its perpetuation. A similar occurrence was reported from Germany by Sorauer (18), mildew appearing on onions grown for seed in April, with the result that seedling onions showed the disease in June; but this worker, any more than the others, did not suspect the presence of perennial mycelium.

Autumn-sown Onions as Carriers of Infection.

Since attention was drawn, in the preliminary note referred to, to the existence of perennial mycelium, observations have been made by many workers in Ireland and England which go to show how commonly the fungus persists by means of perennial mycelium in the bulb, particularly in the case of autumn-sown onions. We are indebted to Mr. A. D. Cotton, formerly Mycologist to the Ministry of Agriculture, England, for communicating to us many observations collected through the medium of the Plant Disease Survey, which he initiated. These have to do largely with hibernation in autumn-sown onions, which our observations, both before and since that time, have shown to be of great importance as disease carriers, if for no other reason than that the practice of growing onions in this way is so common, as the following experiment shows:—

Onion seed was sown in August, 1922, in an open field between two drills of onions on which some mildew was present, and in the late autumn the seedlings were transplanted to another situation. In both cases the ground had not previously been used for onions. No mildew appeared on the seedlings in the autumn, and nothing remarkable was noticed about them until the following spring, when certain plants developed a yellow colour at the tips of the leaves, and in addition showed a general indefinable appearance of ill-health. Two such plants, when examined on April 21st, 1923, were found to be permeated throughout the bulb and leaves with mycelium of *Peronospora Schleideni*, which, as a careful examination showed, had not yet reached the surface and produced conidia. Detailed observation also showed that there had been no onion mildew up to that time in the vicinity, for on the same date another similar infected plant, on which no conidiophores had been produced either, was also found in a neighbouring garden. The disease in both cases is to be traced to infection contracted in the previous autumn, and the observations just recorded were made immediately before the fungus reached the surface for the first time in spring and began spreading to previously healthy plants.

About thirty-six onions survived until spring in the experiment which has been described, and five of these developed suspicious symptoms up to May 31st, and were proved to contain the mildew fungus throughout, the mildew, when it developed, appearing first in characteristic fashion at or near the tip of the oldest leaf (Pl. XIV, fig. 24). The remainder (about thirty-one in number) were allowed to go on until June 13th, when they were individually proved, by covering and subsequent microscopic examination, to be free from internal mycelium and mildew.

Early in May in the same year three further systemically infected autumn-sown onions were picked out in another plot. This was part of an attempt to determine if such cases could be recognised with any accuracy by external appearance—namely, a pale green and glazed appearance of the leaves (particularly the oldest ones) and a yellowing of the tips, denoting general ill-health. Five suspicious cases were selected, of which three were found to be diseased and two healthy, indicating that by the exercise of care and good judgment a considerable amount of infecting material might be removed in April and May. Considerable experience is necessary, however, for the presence of yellow tips on the leaves is by no means confined to plants carrying the mildew fungus. Practical gardeners are often very adept in this work.

Our experience indicates that plants infected in the late summer and autumn may not produce any fructifications during that year, and that the symptoms

of disease produced at that time are generally slight and less easy to recognise than those produced in the late spring. This experience has been an annual one, and was particularly marked in 1926, when a determined effort was made to pick out infected plants in autumn-sown onions at different times during the winter, but without success, for no systemically infected plants could be recognised either by us or by an experienced gardener until May. It may be, however, that some infected plants could also be recognised and eliminated when the seedlings are being removed from frames or seed-beds early in the year (this being a common practice in onion growing); but we have not sufficient experience to say definitely that this can be done.

These conclusions are not to be interpreted as meaning that the disease is not present in autumn-sown plants at an early stage of the season, as the following shows:—Early in February, 1926, some autumn-sown onion plants were submitted for examination, of which four plants out of ten were infected throughout with mildew mycelium. Inquiry showed that they had been grown during the previous summer beside a row of mildewed shallots. It is interesting to note that at a slightly later date the shallots, which were a failure, were dug into the ground, and a late sowing of onion seed was made in September on the site they had occupied, but the plants which were produced in this case entirely escaped infection.

It appears from reports of the Plant Disease Survey (England), before the existence of perennial mycelium was known, that quite obvious symptoms of disease may appear in autumn and early winter (September to December) on seedling onions (6), a fact which is confirmed by Walker (22), and that the earlier the sowing the worse the disease (6 and 1). The observations made in England in 1920 are especially interesting, because no less than seven pathologists in different localities recorded that the disease either began on autumn-sown onions, and in some cases was confined to them, or was worse on them than on spring-sown plants. (Details communicated by A. D. Cotton.) A similar state of affairs was noticed in the years 1918, 1919, 1920, 1921, 1922, 1923, and 1925.

These observations confirm our conclusion that autumn-sown onions are an important source of infection in the spring, and indicate means by which the danger may be reduced in cases in which this system of onion culture must be continued, for there is little doubt that the effect on mildew control would be beneficial if it could be discontinued altogether, particularly where the disease is severe. Sowing the seed in autumn has actually been recommended as a means of controlling the disease, but the practice tends rather in the other direction unless special precautions can be taken to avoid infection in the seedling stage.

The precautions which are advisable are:—(1) To sow the seed as far as possible from other onions, and preferably in fresh soil; (2) to raise the plants under glass in December or January, which experience has shown to be the best way, or, if this is impossible, to sow in the open as late as conditions permit in the autumn, although the exact date when danger may be considered over cannot yet be stated; (3) to examine the plants carefully when they are being transplanted, and particularly at intervals during April and May, and remove any ailing ones, and destroy them, for otherwise they may produce conidia; (4) to sow seed for the spring crop as far as possible from autumn onions.

Onion "Sets," Potato-Onions, Shallots, Seed Onions, and other Cultivated Species of Allium as Carriers of Infection.

Another potential source of infection is to be looked for in onion "sets," which are largely planted in some places, though, as they are very little used in Ireland, we have no direct experience of them. These are onions which are raised from seed sown in spring, but which are not "thinned," so that the plants grow close together and the bulbs remain small. The latter are harvested in the usual way, and are planted in the following season, when, under proper management, large bulbs are produced early in the season. It stands to reason from what has gone before that these plants must also perpetuate the fungus in many cases.

The potato- or underground onion, which is regarded as a variety of the common onion (*Allium cepa*, var. *multiplicans*), is a plant which is propagated vegetatively by planting large bulbs, in the axils of the leaves of which new bulbs are formed, which ultimately break apart; while, by planting small bulbs, large bulbs of marketable size are produced. These onions, which grow to a larger size than, and are distinct from, shallots (*A. ascalonicum*), are commonly grown in Ireland, more particularly perhaps in the north and west, and bulbs for propagation purposes are an article of commerce. The supply is apparently not equal to the demand, largely, it is believed, because the bulbs are very liable to decay in winter, and extremely high prices are paid for them by seedsmen, from a minimum of £1 up to £2 and more per hundredweight. We have found that the bulbs of these onions sometimes harbour mycelium of the mildew fungus, the new bulbs which they produce being also infected from the beginning, and they form another source from which mildew can, and does, spread to ordinary onions. The bulbs in which mildew mycelium was found were not in a sound, firm condition; but we are not in a position to say to what extent this disease by itself is generally responsible for the poor keeping quality of potato-onions, though we believe it plays a part in this.

The shallot (*Allium ascalonicum*) is propagated vegetatively in the same way as potato-onions, and it also has been proved repeatedly to carry the mildew fungus in its bulbs, and to give rise to abundant conidia on its leaves (Pl. XV, fig. 27) in spring. We have proved experimentally that conidia produced on onions can infect the shallot, and it is, therefore, another source of infection for the general onion crop.

Shallots, it is understood, are considered by gardeners to be relatively less susceptible to mildew, but this has not been our experience, so far, at least, as the presence of mycelium is concerned, whatever may be the resistance after infection is contracted. Severe attacks on this crop are also reported by Gram and Rostrup (9) in Denmark, and frequent attacks were noted in England in 1920, although there also the crop is regarded as highly resistant. Attacks on shallots have also been recorded in other countries. The fact that all the plants grown commercially as "shallots" do not belong to *A. ascalonicum*, some being apparently varieties of *A. cepa*, may account for the differences in susceptibility noted.

There remains the question of onions which are planted for the production of seed, which, however, is of little or no importance in Ireland. It has been proved by direct experiment that by planting affected bulbs the mildew may grow up in the leaves and flowering shoots, and fructify on them in early spring. If the bulbs are very badly diseased, no flowering shoot may be formed, or the

shoot may develop conspicuous yellow spots and fall over before the flower matures, and, as a rule, no seed is produced; but less badly affected ones, and those belonging to more resistant varieties, may flower and seed almost normally. It may be stated that seed saved from two lots of plants of this description was sown beside control lots of seed from healthy plants, but there was no evidence that infection was carried in this way, for all the plants remained healthy.[1]

The only species of *Allium* on which mildew is generally recorded as occurring are those given by de Bary (7), *A cepa* and *A. fistulosum*. Some authors add "and on other species of *Allium*," while *A. sativum* (garlic) is given as a host in a few cases. The disease is definitely recorded on this plant by Caballero (3). It has also been reported on the shallot (*A. ascalonicum*) in many countries, as previously stated. The leek (*A. porrum*) was recorded as a host as long ago as 1899 by Schöyen (15), again in 1912 by Voglino (21), and lately by Gram and Rostrup (9), as well as in England (1). Nevertheless, this occurrence is rare, and we have not found the disease on leeks in Ireland. The Egyptian or tree-onion (*A. cepa* var. *bulbelliferum*) is, however, fairly readily attacked here. This plant is not unlike the potato-onion, and appears to be sold sometimes for it; but, in addition to propagating by division of the bulbs, it also produces bulbils and seed. The reaction to the disease of the other cultivated species, *A. scorodoprasum* (sand leek or rocambole) and *A. schoenoprasum* (chive), does not appear to be known.

Before these plants can be recognised as a source of infection for the onion crop, it is necessary to know whether conidia from one plant will infect a plant of another species. Our experiments, so far as they have gone, show that the fungus from *A. cepa* readily attacks *A. ascalonicum* and *A. cepa* var. *bulbelliferum*, but under the same conditions *A. porrum*, *A. fistulosum*, *A. sativum*, *A. scorodoprasum*, and *A. schoenoprasum* did not contract the disease.

Spread of the Disease from Systemically Infected Onion Plants in early Summer.

The first outbreaks on spring-sown onions, which under our conditions were generally grown in the neighbourhood of autumn-sown plants and other proved (or possible) sources of infection, were generally observed early in June. It has been proved that when the first fructifications appear on the surface at this stage, the mycelium is already present in most of the green leaves, scale leaves, stem, and (sometimes) the young leaves developing within the bulb, but with the exception, as a rule, of the tip of the oldest leaf, in the base of which the fungus is present. In the light of our experience with the disease, this indicates recent infection through the green portion of one of the oldest leaves, the time of infection being probably three to four weeks previously, or some time between the first and middle of May. This conclusion agrees with previously recorded observations on the date of appearance of the first fructifications on systemically infected autumn-sown plants, from which the infection most likely came.[2] The

[1] Chapman (4) records the presence of spores of *P. Schleideni* on onion seed, but the original paper was not available, and an inquiry addressed to the Massachusetts Agricultural Experiment Station elicited no reply. These spores can only have been resting spores, because the conidia would not survive; and it is probably to this work that Chupp (5) refers when he says (p. 287) that "an examination of the seed has revealed an admixture of oospores."

[2] Infection from the soil will suggest itself, but contemporary experiments with soil from the plots on which these notes were made failed to produce infection in young plants, as already stated. Jones (10, 11) demonstrated the existence of soil infection, but in this case resting spores were produced abundantly.

first appearance of conidia in any quantity on such plants may be expected, as a rule, early in May or late in April, although it has exceptionally been noted in favoured spots in the open as early as 24th February.

The behaviour of the mycelium when it has entered a plant varies according to conditions, for in some cases it spreads rapidly through the plant, without fructifying at first, with the result that the first mildew to appear may be far distant from the original point of infection. In other cases, particularly as the season advances, fructifications are rapidly produced on the site of the original infection centre, and while the disease is localised. Still other cases are a combination of these two conditions. The following summary of the distribution of mildew mycelium in July in a plant derived from seed sown in spring is typical :—

Leaf No.	Length.	Mycelium in— Scale leaf.	Mycelium in— Green leaf.	Mildew fructification.	Remarks.
1 (outermost)	16 cm. (tip gone)	+	–	–	
2	38 cm.	+	+ near base. – above.	+ near base.	Original infection from which bulb became diseased.
3	48 cm.	–	+ at centre. – above and below.	+ at centre.	Independent aerial infection.
4	39 cm.	–	+ ⅔ way up. – above and below.	+ (slight) ⅔ way up.	Independent aerial infection.
5	18 cm.	–	–	–	
6	5 cm.	+	–	–	
7	> 1 cm.	+	(None.)		
Stem.			Mycelium present.		

The original infection took place near the base of No. 2 leaf, from which point the mycelium spread downwards (much more so than upwards) into the scale leaf and stem, and from that into the bases of the youngest leaves, missing the bases of three intervening leaves, temporarily at least. A preference for the young and actively growing leaves was also noted on other occasions, but in most cases, perhaps, the bases of all leaves are invaded in order. The mildew was fructifying on the original infection centre. Two other independent infection centres were also present on leaves Nos. 3 and 4, on which the fungus was also fructifying, but from which the mycelium had not yet spread very far. It will be noted that comparatively young leaves, as well as older ones, are capable of being infected by conidia (leaves up to the fifth in order from the outside have been noted as infected at this stage), but fresh aerial infections are not usually evident on the youngest leaves.

A study of these facts shows how difficult the disease is to deal with by spraying, for, apart from other considerations, once a single leaf is penetrated the fungus has the whole plant at its mercy, and can produce its fructifications in any place at the favourable moment. To be successful, spraying would have

to be begun about May 1st, and be continued at intervals of probably three weeks or one month until a short time before the bulbs are harvested, for, although late infections might do little harm to the leaves, their effect on the keeping quality of the bulbs might be disastrous. As many as five or six applications of spray in the season would probably be necessary for spring-sown onions which are harvested in September, and about four for autumn-sown plants. No spraying experiments were carried out by us, but the foregoing considerations will have to form the basis of any successful fungicidal treatment; and the comparative failure of previous attempts in this direction is probably to be attributed less to the difficulty of getting sprays to spread on, and adhere to, the cuticle than to want of knowledge of the inter-relations of parasite and host.

Resistance of Onion Varieties to Mildew.

It appears to us that the varying resistance of varieties of onions to this disease offers a useful line of attack. Observations in 1922 on a restricted number of varieties in one field, where they were growing side by side under comparable conditions, showed that there were marked differences in this respect, Up-to-Date being the most resistant, followed in order by Stirling Exhibition, All-the-Year-Round, and an American variety, Wethersfield Red, which was clearly the worst. It is interesting to note that the keeping quality of the bulbs followed the same lines. By early December the varieties Wethersfield Red, All-the-Year-Round, and Stirling Exhibition were almost a total failure, the bulbs having sprouted and become soft, and mildew mycelium being found in practically every one examined. Up-to-Date kept very much better, there being only twelve sprouting bulbs out of about 2 cwt. at that time, a proportion which had increased to about one in eight in February, when the sprouting bulbs were generally found to contain mildew mycelium, while the firm bulbs were free from it.

More extensive observations on a larger number of varieties growing side by side in 1923 confirmed the existence of differences in susceptibility. On September 6th the following percentages of plants were entirely killed by mildew; but it must be stated that practically every one of the remainder, if not every one, was attacked to some extent. The plants had not been sprayed:—

Ailsa Craig ...	... 20 per cent.	All-the-Year-Round ...	... 70 per cent.
Webb's Reliance ...	... 25 „	Magnum Bonum ...	... „ „
Webb's Ringleader ...	... „ „	Sutton's Long Keeping ...	„ „
Cranston's Excelsior	... „ „	Sutton's Improved Reading ...	80 „
The Globe (Sutton) ...	... 40 „	Webb's Improved Banbury ...	90 „
Bedfordshire Champion	... „ „	Stirling Exhibition ...	... „ „
Rousham Park Hero	... 50 „	James's Keeping* ...	... „ „
Up-to-Date (Lot 1) ...	... „ „	Sutton's A. 1 ...	... „ „
„ (Lot 2) ...	... 56 „		

* *Very few plants of this variety.*

Observations were not made on the keeping quality of the bulbs in this case.

Treatment of Infected Bulbs by Dry Heat.

Following preliminary trials, in which both bulbs and mycelium were killed, a series of experiments was initiated, in which infected bulbs were submitted to dry heat of varying degrees of intensity, in the hope of finding a treatment

which would kill the mycelium without injuring the bulb. Five consecutive experiments were conducted, gradually decreasing temperatures being employed when it was found that the treatment used in the previous experiment had been sufficient or more than sufficient. The experiments extended from November, 1925, to March, 1926, and the results of all can be conveniently presented together, as is done in the following table:—

Temperature used. ° C.	Duration of treatment. Hours.	Limits of weight of bulbs. Grams.	No. of bulbs which— were treated.	grew.	No. of bulbs in which mycelium— survived.	was killed.
52°	8	27–68	3	0	—	—
	4	49–91	3	0	—	—
49°	8	20–71	3	0	—	—
	4	21–99	3	1	—	1
47°	8	—	3	0	—	—
	4	—	3	1	—	1
45°	8	50–113	3	2	—	2
	4	42–65	3	2	1	1
43°	16	72–133	3	3	—	3
	8	56–111	3	2	—	2
41·5°	8	58–90	3	2	—	2
	4	53–70	3	3	3	—
40°	24	77–126	3	3	—	3
	16	59–98	3	3	—	3
	8	61–120	3	3	—	3
38°	16	70–93	3	2	—	2
	8	67–90	3	3	2	1
36°	16	66–89	3	2	1	1
	8	55–97	3	3	2	1
Controls combined (not heated) ...			9	7	7	—

The results may be summarised as follows:—Treatment at 40° C. for 8, 16, and 24 hours, or at 38° C. for 16 hours, was sufficient to kill all the mycelium in the bulb without causing apparent injury to the host. A lower temperature than these, or a shorter period at 38° C., was not effective. Higher temperatures than 40° C. were generally effective, as would be expected, except in several cases where the duration of treatment was only 4 hours. These cases were very interesting, for examination carried out soon after treatment showed that the mycelium which was already in the leaves (whether these were still within the bulb or had emerged) had been killed by the heat, but that the shoots were being re-invaded from the root-stock, in which the fungus was not killed. Eventually these plants again became diseased throughout. This occurrence was fortunate, as it showed that the arbitrarily chosen minimum of 4 hours was not far from the mark, and yet not sufficient to allow the heat to penetrate into the more solid base of the bulb. On the other hand, 8 hours seem to be sufficient for this at a temperature of 40° C., although much longer periods caused no apparent injury.

The treated bulbs, which were planted immediately after treatment, were kept under observation for not less than 61 days, unless mildew developed sooner, and in the case of the more critical experiments at 40° C. and upwards, for at least 76 days. During this period obvious mildew appeared on all the surviving controls, and on the plants heated at 36°–38° C., in which the fungus was not killed, and complete re-invasion of the plants which were kept at higher temperatures for 4 hours also took place. No mildew appeared on the remaining plants after being kept covered for 9 days, and microscopical examination of every leaf proved that the fungus was entirely dead.

This experiment points to the probability that in the case of onion bulbs intended for planting the disease can be prevented by dry heat at 40° C. for about 8 hours, though the duration would probably vary with the size of the bulbs. The weight of the bulbs used in our experiments is shown in the table referred to. We have not tried shallots or potato-onions, but in all probability they will be found to behave similarly. A form of treatment is also required which can be applied in autumn, so as to safeguard bulbs intended for culinary purposes during the winter; but we are not yet in a position to say whether the procedure just suggested would be safe in this case, as it appears to have a tendency to cause the bulbs to sprout. It is hoped to carry out further experiments on this point.

Attempts to Grow the Fungus on Artificial Media.

All attempts that have been made to induce the mildew fungus to grow in culture on natural or artificial media have ended in failure. The fact that the fungus does not grow out of infected scale leaves, as has been proved repeatedly, has already been referred to, and this no doubt helps to explain the failure of cultures and infection experiments using this material as a starting point. Thus in one set of cultures, in which portions of infected scales, taken out with the usual precautions against contamination, were placed on the surface of oat-agar slants, examination after sixteen days showed that the mycelium in the tissues had made no progress, and was less vigorous than at the beginning. The only development ever noticed was the formation of round bodies, which might be taken for incipient oogonia, but which never develop far enough to be definitely recognised. No attempts at the formation of antheridia in connection with these bodies were ever seen, and no conidiophores were ever produced. The following substrata were used in connection with attempted cultures from scale leaves:—Scale leaves, green leaves, sterile uncooked onion extract, onion extract agar, potato agar, and oat agar made from various forms of oat-meal. Green leaves containing intercellular mycelium were, in addition, used with some of these media, but with no success throughout.

Cultures originating from conidia were hardly less unsuccessful, for, when the conidia were transferred under aseptic conditions from leaves to set plates of oat agar and onion extract agar, they germinated indeed on the oat agar, but did not develop beyond the germ-tube stage, although the plates were very free from bacteria and moulds. Some of the germinated conidia were picked off the plate and transferred to hanging drops of water and sterile uncooked onion extract, but no further development took place up to eight days afterwards. Practically no attempt whatever at growth was made on onion extract agar, or in the liquid extract without agar.

Summary.

The occurrence is recorded in the bulbs of onions (*Allium cepa*), potato-onions (*A. cepa* var. *multiplicans*), shallots (*A. ascalonicum*), and Egyptian onions (*A. cepa* var. *bulbelliferum*) of a perennial mycelium, which was proved to be that of *Peronospora Schleideni*, and which was subsequently found to permeate the green shoots which such bulbs produced.

The effect of the disease on the bulbs and the development of diseased shoots and subsequent production of mildew on their surface are described.

Onions were infected with mildew through the leaves, and the mycelium was then found to spread down from them into the bulbs.

Attempts to inoculate onion bulbs directly, whether growing in soil or otherwise, failed, infections only succeeding through the leaves.

Resting-spores were found occasionally in the leaves of mildewed plants, but their occurrence is very rare in Ireland, where soil infection appears to be practically non-existent.

Perennial mycelium is, on the other hand, extremely common in Ireland, where it is believed to originate most of the disease, and evidence is presented pointing to its probable occurrence elsewhere.

Onions raised from seed sown in autumn were repeatedly proved to harbour mycelium of the mildew fungus during the winter, and to produce the earliest outbreaks of mildew on their leaves in the following spring. The importance of these plants as centres of infection is emphasised, and means for eliminating danger from them are outlined.

Onion "sets," potato-onions, shallots, onions grown for seed, Egyptian onions, and possibly other species of *Allium* are also capable of carrying the fungus in their bulbs and originating the disease in spring.

The infection of onions raised from seed in the spring from such systemically infected plants has been followed. No evidence of infection of the seed was secured.

Notes are given on the varying degrees of resistance of the foliage and bulbs of some varieties of onion to mildew.

Heating affected onion bulbs in air at 40° C. for eight hours was effective in destroying the mycelium.

Attempts to grow the mildew fungus on artificial media were unsuccessful.

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DESCRIPTION OF PLATES.

All Figures are magnified 300 times, unless otherwise stated.

Plate XII.

Fig.

1. Mycelium of *Peronospora Schleideni* among thick-walled cells of stem, or root-stock, of onion.
2. Mycelium in meristematic tissue of root-stock.
3. Mycelium growing up from root-stock into base of young leaf. Plants sown 4/7/'23, artificially infected through oldest leaves 30/7/'23, examined 24/8/'23. See p. 245.
4. Long straight hypha of *Peronospora Schleideni,* with haustoria, growing up between elongated cells near the inner surface of the leaf.
5. Mycelium and haustoria in old scale-leaf of onion. × 120.

6 & 7. Mycelium at base of young developing leaves.

8. Longitudinal section of portion of leaf, showing position of mycelium during earlier stages of invasion, just inside more specialised assimilating parenchyma. × 180.
9. Transverse section of a leaf, showing distribution of mycelium in inner tissues at same stage as shown in fig. 8. × 180.
10. Trifurcate haustorium from leaf.

Plate XIII.

11. Invasion of spongy and palisade parenchyma of leaf by mycelium of *Peronospora Schleideni,* preparatory to formation of conidiophores. × 200.
12. Very stout hyphae of irregular shape, and densely filled with protoplasm, dissected out from assimilating tissue of leaf.
13. Mycelium with a haustorium in a palisade cell.
14. Fragment of conidiophore showing early stage in the formation of conidia.
15. Later stage in the development of conidia.
16. Conidiophore of *Peronospora Schleideni.* Somewhat diagrammatic. × 180.
17. Conidia.
18. Early stages of germination of conidia in tap water.
19. Further stages of germination in tap water and boiled onion extract (in centre).
20. Regularly coiled germ-tube produced in deep layer of water.
21. Germination in soil extract, showing maximum development of germ-tube observed.
22. Sexual organs, some perfect and others more or less abortive, from base of leaf of mildewed plant in region of neck of bulb. The two larger oogonia in bottom left-hand corner occurred together, and appear to be furnished with a single antheridium. To the right, oogonium with oospore.

PLATE XIV.

23. Development of mildew on leaves of shallot nineteen days after planting bulb which contained mycelium of *P. Schleideni*. See p. 239.

24. Typical appearance of mildew in spring near tip of oldest leaf of systemically infected autumn-sown onion.

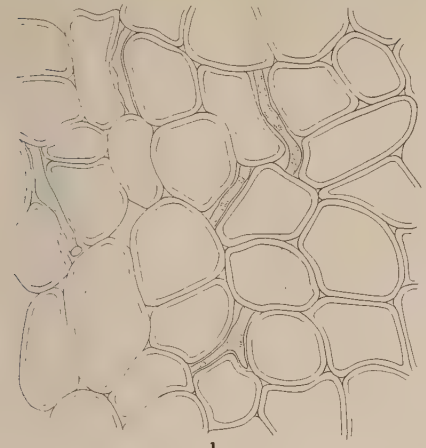
25. Mildew on flowering shoot produced by onion bulb which contained non-septate mycelium. It was by means of this plant that the connection between this mycelium and *P. Schleideni* was first proved. See p. 238.

26. Typical appearance of diseased (right) and healthy (left) onion bulbs in storage. Variety Up-to-Date.

PLATE XV.

27. Appearance of systemically infected shallot, grown in the open, after mildew appeared on 4/5/'21.

28. Effect of heat treatment of diseased onion bulbs. Three plants on right are produce of infected bulbs heated at 40° C. for sixteen hours, and are entirely healthy. The bulbs which produced the three plants on left, which have been killed by mildew, were treated at 38° C. for eight hours, which was not sufficient to kill the mycelium.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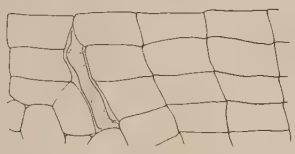
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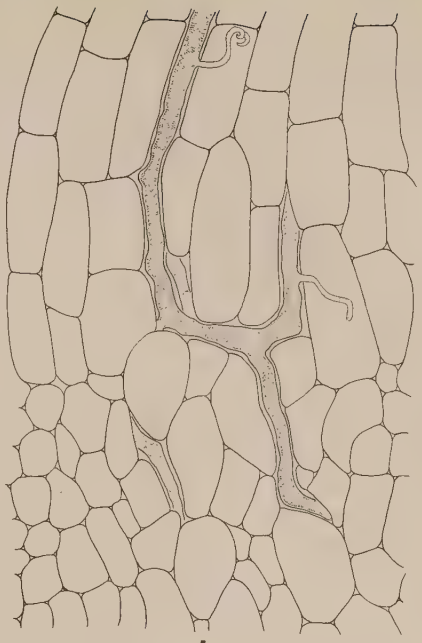
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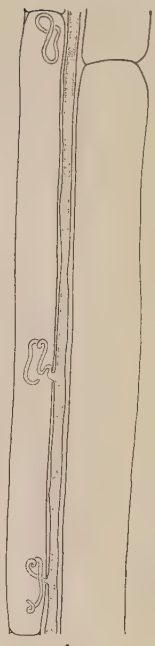
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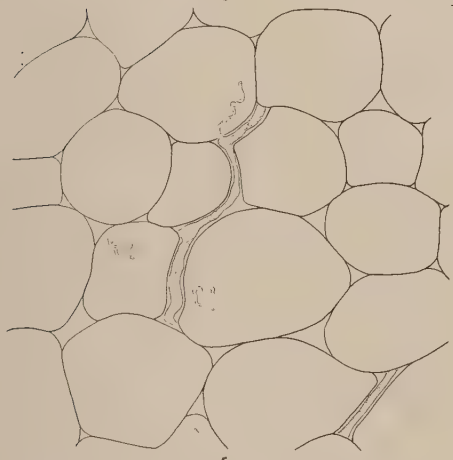
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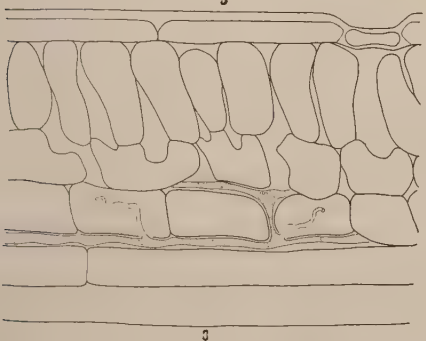
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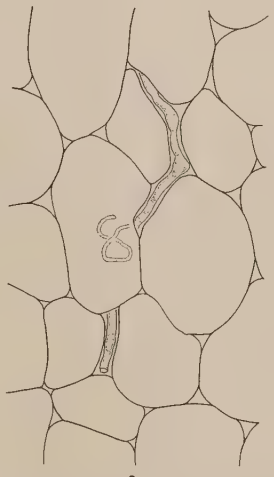
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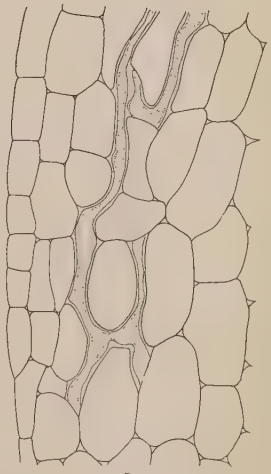
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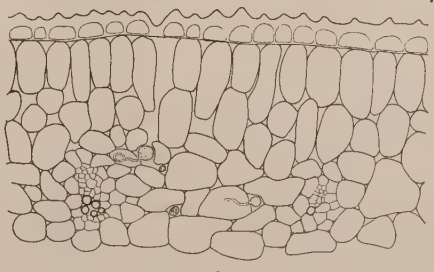
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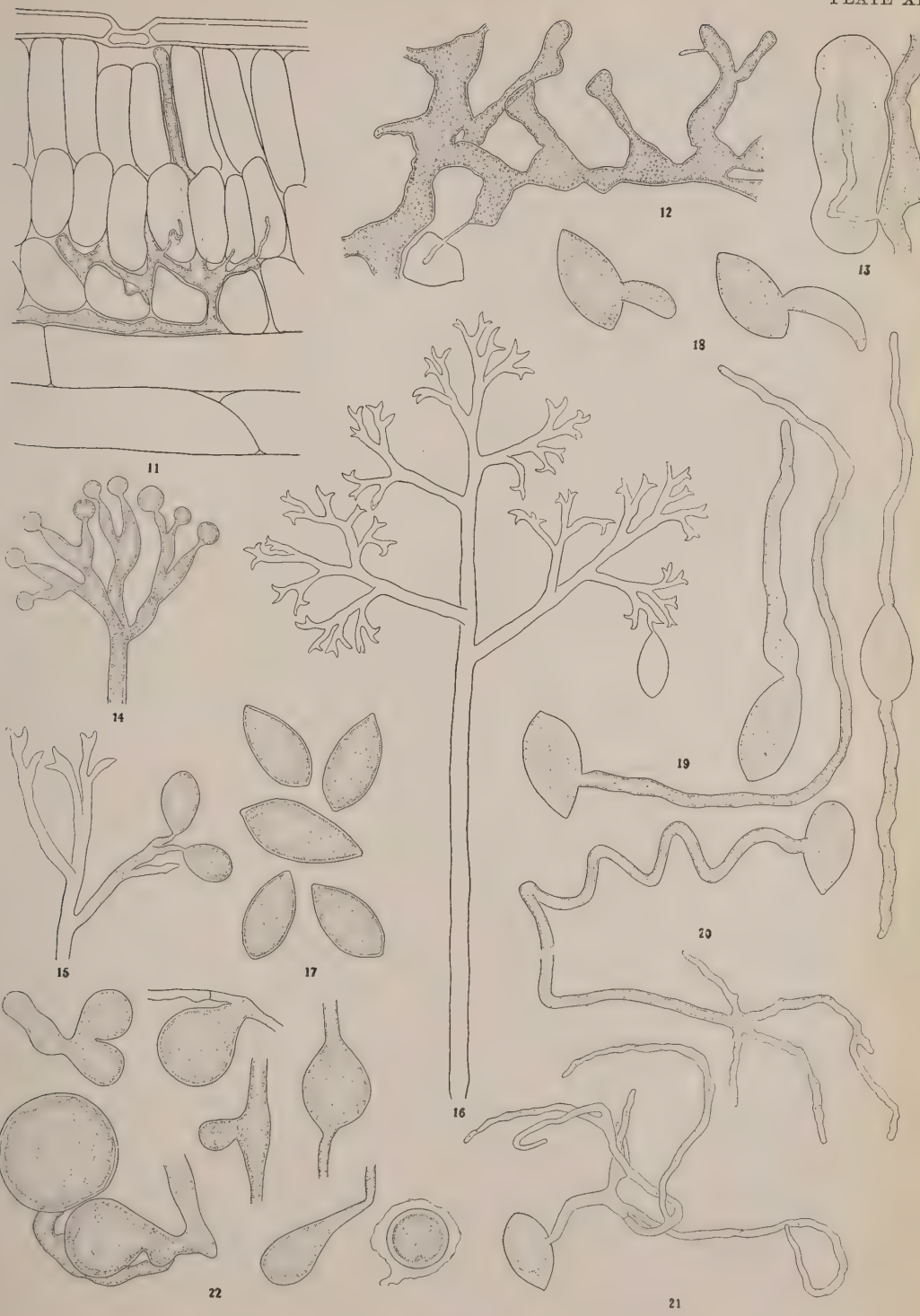
FIGS. 1–10.



FIGS. 11–22.

FIG. 23.

FIG. 24.

FIG. 25.

FIG. 26.

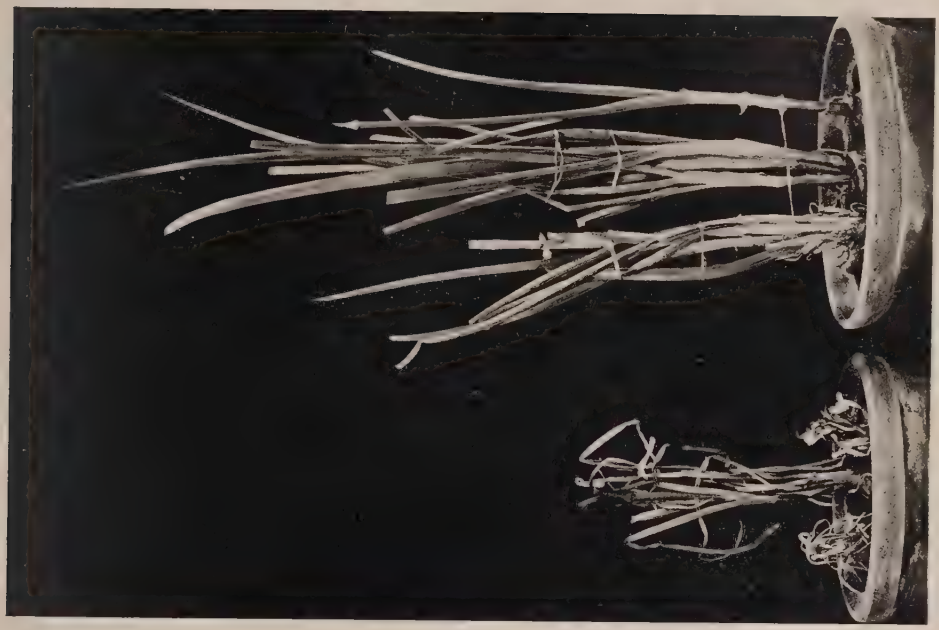


FIG. 28.

FIG. 27.

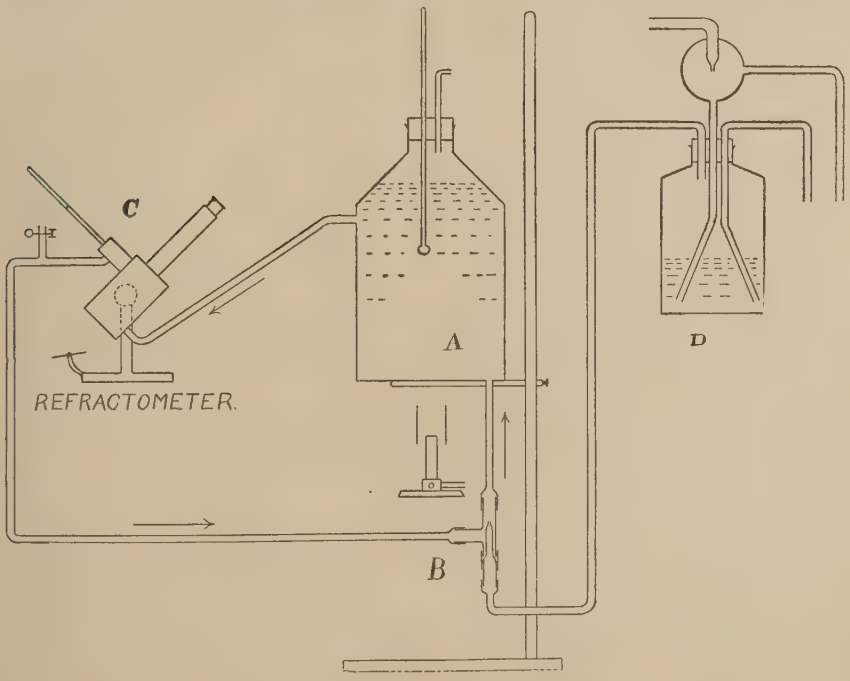
No. 23.

A SIMPLE METHOD OF TEMPERATURE CONTROL FOR USE WITH REFRACTOMETERS AND POLARIMETERS.

By MICHAEL T. CASEY, M.Sc., State Laboratory, Dublin.

[Read April 27. Published separately July 30, 1926.]

The ordinary heating apparatus, supplied with refractometers, works satisfactorily only when used regularly and over prolonged periods. Where the number of observations to be taken is small, it is found that this apparatus is rather elaborate, and requires considerable manipulation in order to maintain the desired temperature. The apparatus shown in the appended diagram was designed to avoid this difficulty.



The refractometer prisms are kept at the required temperature by means of a current of water circulated from a reservoir (A), which consists of a copper

can capable of holding about three litres. The can carries two copper tubes having an internal diameter of three millimetres. One tube is placed about three centimetres below the top of the cylindrical side, the other passes through the base at the same distance from the opposite side. To the lower tube is attached a modified filter-pump (B), consisting of a **T**-piece holding a glass tube drawn out to a moderately fine jet at approximately one centimetre above the junction of the side tube of the **T**. The apparatus is mounted on a retort-stand, and adjusted so that the upper or exit tube of the reservoir, when connected with the inlet of the refractometer, is about ten centimetres above the level of the prisms. The outlet tube of the refractometer is connected with the side tube of (B) through an inverted **T**-piece fitted with rubber tube and a spring clip.

The reservoir (A) is now filled, to within seven centimetres of the top, with water. The clip on the inverted **T**-piece is opened to allow the air to escape from the tubes, and the whole apparatus becomes filled with water.

The central tube of (B) is connected with an air-blast, which may be conveniently obtained by adapting a laboratory filter-pump to the well-known device shown at (D). The air emerging from the jet at (B) forces the surrounding water to ascend into the reservoir, and more water is drawn in through the side tube. In this way a continuous circulation is maintained. The water in the reservoir is heated by means of a Bunsen burner, the flame of which is shielded from draughts. When the thermometer at (C) registers the required temperature, the flame is reduced and observations may be taken with the refractometer.

Advantages which may be cited in favour of this heating apparatus are the following:—

(1) Cheapness and simplicity of construction; its total cost is approximately one-tenth that of the more elaborate apparatus.

(2) Facility of adjustment of temperature, even over prolonged periods of working.

(3) Minimum loss of heat, since the warm water leaving the refractometer is returned to the reservoir, slightly heated and used over again, instead of being discharged into the sink as in the case of the heating apparatus supplied with the instrument.

In conclusion, I have to express my sincere thanks to Professor Hugh Ryan for his kind criticism, and also for permission to publish this paper.

No. 24.

THE DEHYDRATION RATES OF CONIFER LEAVES IN RELATION TO PENTOSAN CONTENT.

By JOSEPH DOYLE,
Professor of Botany, University College, Dublin,

AND

PHYLLIS CLINCH, M.Sc.,
Demonstrator in Botany, University College, Dublin.

[Read April 27. Published separately July 30, 1926.]

In a previous communication (4) attention was drawn to a theory of cold-resistance recently propounded by Rosa (8). The main point of this theory is, that death from freezing is due to the disturbance of the water balance in the cell; and, if there are present, presumably in the protoplasm, substances that have marked powers of imbibition, they may retain sufficient water against the tendency to ice-formation to prevent the physico-chemical disarranging of the protoplasmic structure which results in death. Such substances he recognizes in hydrophilous colloids, especially the pentosans; and he, therefore, relates the gradual development of cold-resistance in hardened plants, like cabbage, to an increased development of pentosans, which he observes in these plants during a process of hardening. In our first communication, we examined a limited number of Conifers from the point of view of this pentosan theory, and could find no relation between the hardiness of the forms examined and the relative pentosan content, or between pentosan development and the change from summer through autumn to winter conditions. It was found that the pentosans could be divided into three classes—(*a*) pentosans extractable with hot water only, (*b*) pentosans subsequently extractable with 1 per cent. HCl, and (*c*) pentosans extractable from the residue by 12 per cent. HCl. Now, Rosa's pentosan increase during the hardening process of his plants was almost entirely in the water-soluble fraction. This fact can obviously be interpreted in favour of his theory.

Rosa, however, went further. Though recognizing the vexed question of the existence of any connection between drought-resistance and cold-resistance, yet, impressed by the importance of imbibitional forces in the cell, as brought out by work such as that of McDougal and his school, he lays stress on the comparative drying rates of the leaves of the hardened and unhardened specimens of the cabbage and tomato plants with which he dealt. Arguing that, if these pentosans can imbibe water, and can, therefore, hold some of it against the tendency to ice-formation, they should also be capable of holding it against forces of dehydration, he compared the transpiration rates and rates of drying of hardened and non-hardened plants, finding in every case a decreased rate both of transpiration and of drying in the hardened forms, thus further supporting his hypothesis. In a later separate contribution, Boswell (2) gives data and curves derived from Rosa's plants, and is in full agreement with

him that the hardier plants dried at a slower rate than the less hardy. In fact, he even concludes that the method of drying may be used to estimate the relative amounts of free and unfree water in plant tissues, and that the comparative rates of water loss, under uniform conditions, are a measure of the relative hardiness of certain kinds of plant tissues. As so simple a test might be of great value in horticultural practice, it seemed advisable, apart from the pentosan question, to see how the drying rates of the Conifers bore upon it.

The method chosen by Rosa and Boswell was rather rough, as the leaves were merely dried in a hot oven at 60° C. Although we felt that many interesting points in the drying rates of plant tissues are completely overlooked in such rapid dehydration, we decided, in order to parallel our results as closely as possible with theirs, to use the same temperature. The plants used for the dryings were:—

Pinus larcio v. *nigricans* Parl. (Austrian Pine.)
Pinus montana v. *uncinata* Willk. (Mountain Pine.)
Tsuga Albertiana Sén. (Western Hemlock.)
Abies pectinata De C. (Silver Fir.)
Cupressus Lawsoniania Murr. (Lawson's Cypress.)
Larix europoea. (European Larch.)

With the exception of *Pinus montana*, they were all from trees in the Botanic Gardens, Glasnevin. *Pinus montana* was secured from the Department's Forestry Station at Avondale, Co. Wicklow. The Glasnevin plants were transferred as soon as cut into a small bag, well packed with barely moist cloth, and carried by cycle to the laboratory, no proper facilities for drying being available in the Gardens. We may say here that, especially during the winter, in which season most of our published results were made, there was only a negligible change in water-content during the journey. If, on reaching the laboratory, there was prospect of even a slight delay, the shoots were kept with their stems in water. Such precautions could not be taken with *Pinus montana*, and, accordingly, we do not lay any stress on that form, though when well packed and in large quantity, the few hours' delay between cutting and delivery in no way affected agreement with the other forms. On arrival at the laboratory the leaves were dried on weighed gauze trays, just as was done by Boswell, in a water oven, with a small top opening, kept at 60° C. The needle leaves of these Conifers allowed a considerable quantity to be used with minimum covering of one another. Departure of data from the normal is probably almost entirely due to such slight covering. Four sets of leaves on four trays were dried together, thus allowing an interval for each set of twenty minutes between each weighing. The trays were removed from the oven, allowed to cool, weighed, and then replaced. This was repeated till two consecutive weighings showed no difference, when they were transferred to a 100° C. oven till the last traces of water were removed. From this final weight the water content was calculated. This is, of course, rough and ready, but no more so than Boswell's method, and certainly more accurate than endeavouring to place the leaves for each weighing into a weighing bottle and then back to the tray. A much greater error is introduced in that way, because not only is exposure to the air almost as long, but the loss of small pieces of leaf is unavoidable, and such loss is often considerable, especially towards the end, when the leaves are brittle. In other words, we are emphasizing the fact that, for investigations into the real problems of tissue dehydration, our method was very crude, but, for comparison with Boswell's and Rosa's results, it was sufficiently refined.

Two sources of error of unknown magnitude, to which, however, attention must be called, are—(1) possible oxidations during the drying, (2) loss of volatile materials from the leaves. Further, the leaves are killed very early in the drying. Drying records were made at frequent intervals throughout the year. Upwards of 200 estimations were made, but as a general agreement—indeed, an almost universally close agreement, in view of the method—ran through them all, we have contented ourselves with calling attention to a few of the results only. They are plotted on graphs from calculations showing the percentage of total water present at the various intervals. As different plants had a different water content, results based on a percentage of the total fresh weight of the leaves were not so readily comparable for our present purposes. We might submit the curves of any period of the year, but choose to submit a few relating to the winter period. Now, during this period, we have a close agreement between the Austrian Pine, the Hemlock, and the Mountain Pine, all three showing a very low minimum content of water-soluble pentosan; while, in contrast, Lawson's Cypress showed a remarkable increase in this fraction from October, a high maximum being reached in January. This maximum is actually greater than the recorded maxima of other forms at any period of the year. If, as Rosa suggests, the dehydration rates are in relation to the quantity of water-soluble pentosan present, we should see the relationship in the records of the winter months; while, even if we merely agreed with Boswell that the rate of water loss under uniform conditions is an index of hardiness, the winter records should supply the evidence. The rates here considered are, therefore, those for Cupressus in October, late November, and mid-January; and for the Austrian Pine and *Tsuga albertiana* in late November. Since Rosa related his drying rates to the amount of water-soluble pentosan present, it is as well to list here the corresponding amount of water-soluble pentosan present in the named forms for the period cited. It is expressed as percentage of the dry weight:—

	Oct.	Nov.	Jan.
Cupressus	1·69	2·30	5·06
Tsuga	—	·45	—
Austrian Pine	—	·55	—

The differences here are much greater than those ordinarily found by Rosa, the variation in the experiment from which he draws his graph to show the increase in pentosan development during the hardening process being from 2·97 per cent. to 5.84 per cent. of the dry weight.

We can now turn to the graphs. In each case the curve stops at the point at which practically constant weight was attained in the 60° C. oven. Fig. 1 shows the curves obtained by plotting the percentage of the total water remaining as ordinate against the time in minutes as abscissa, for Cupressus for October and January. The record for late November, we felt, was better omitted from the actual graph. It coincided so closely with the January record that a very great increase in scale would be necessary to demonstrate graphically any difference between them. We are justified, surely, in claiming that no appreciable difference can be maintained between them. In fact, the experimental error of the method has worked out in such a way that the October specimens with least pentosan content are actually recorded as drying more slowly than the January specimens with high pentosan content. In these cases, therefore, no relation can be substantiated between rates of drying and pentosan content. Further, if a sufficient number of points were available to give in each case an undoubted smooth curve, we seem to find suggested an even rate of loss

to a critical point in the neighbourhood of 4 per cent. or less, at which point all the free water has been got rid of, leaving only the water, hygroscopic, absorbed, chemically bound, whatever one chooses to call it, which is in relation to the minute structure of the wall fabric of the cells. In the drying of wool, for instance, it is interesting to note that Fischer (5, 6) records about the same percentage (5 per cent.) of closely bound water. But as we really have not

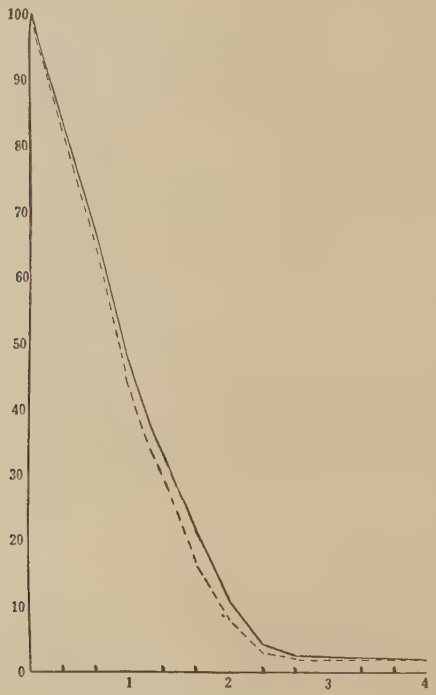


Fig. 1.

Rate of water loss from Cupressus in January (broken line) and in October (continuous line). Ordinate = per cent. of total water remaining at each successive period. Abscissa = time in hours, the intermediate dots representing the 20-minute intervals at which the weighings were made.

data here to analyse these drying rates thoroughly, we can pass on to Fig. 2. Plotted in the same way as the others, it includes the drying rate of the Austrian Pine, Tsuga, and Cupressus, all for late November. Here, though Tsuga is much poorer in pentosan content than Cupressus, it dries slightly more slowly; while the Austrian Pine, whose pentosan content is little or nothing, dries at a very slow rate indeed. Now it cannot be claimed that the Austrian Pine in late November is to any extent at all hardier than Cupressus. It would appear then that even Boswell's claim, that the rate of water loss under uniform conditions can be taken as an index of hardiness, is not substantiated. If hardened forms do actually dry more slowly than non-hardened it is not an

index of their hardiness, but of other changes which have taken place in them as a result of the particular type of hardening process employed. Now in the earlier stages of the work, before the interest of the water-soluble fraction was fully appreciated, but while it was clear that the value of the total of all pentosans present was relatively a little high in the Austrian Pine, curves such as this gave the impression that the pentosan content might perhaps be effective, because at any season of the year the relative drying rates of the Austrian Pine and any of the other forms except *Pinus montana* are similar to that shown in

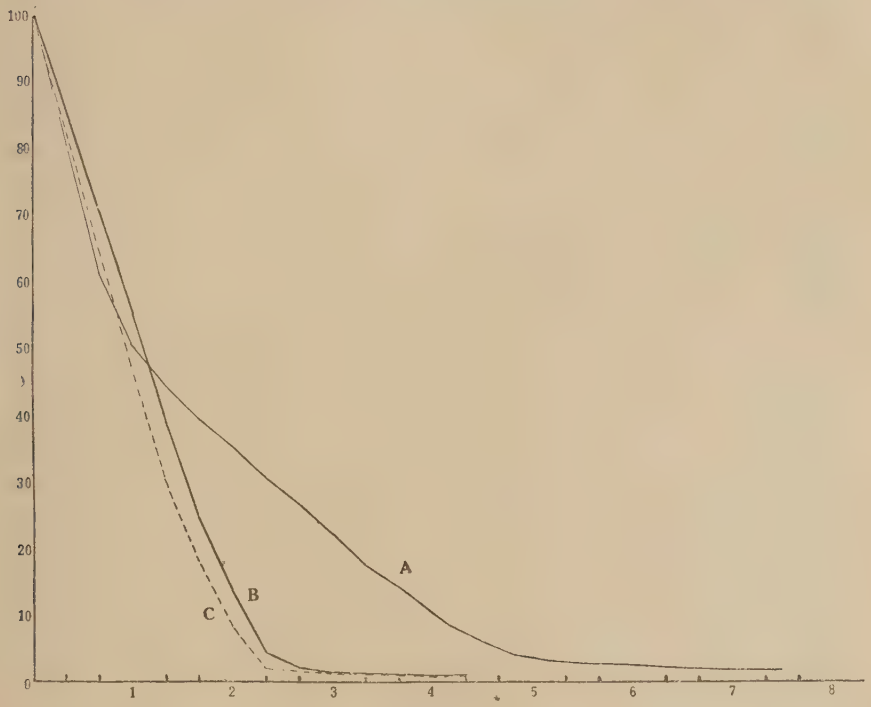


FIG. 2.

Rates of water loss in November from Austrian Pine A, Tsuga B, and Cupressus C. Ordinates as in Fig. 1.

Fig. 2. *Pinus montana*, we may here note, though not quite identical, has a slow rate of drying, similar to that of the Austrian Pine. But if we put down the November analysis of the three pentosan fractions it will be obvious that the drying rate of the Austrian Pine bears no relation to any of them.

		W.S.	1 p.c. HCl.	T.L.	12 p.c. HCl.	G. Total.
Cupressus (Nov.)	...	2·3	4·8	7·1	·97	8·07
Austrian Pine	...	·55	5·5	6·05	1·4	7·45
Tsuga	...	·55	4·26	4·81	·14	4·95
Cupressus (Jan.)	...	5·06	1·25	6·31	1·08	7·39

T.L. = sum of the water-soluble fraction and the fraction extractable by 1 per cent. HCl., and is referred to as the total of loosely combined pentosan.

The Austrian Pine certainly has a slight increase in the 1 per cent. HCl. fraction, but that can hardly be of much importance, since the T.L. fraction of Cupressus is higher still. So it gradually seemed obvious that the marked difference in the drying rates of the Austrian Pine and the others was solely due to its mechanical, xerophytic structure. If so, leaves of the Austrian Pine, if finely yet rapidly chopped and then dried, should closely approximate to a form like Tsuga or Cupressus chopped in the same manner. Further, if

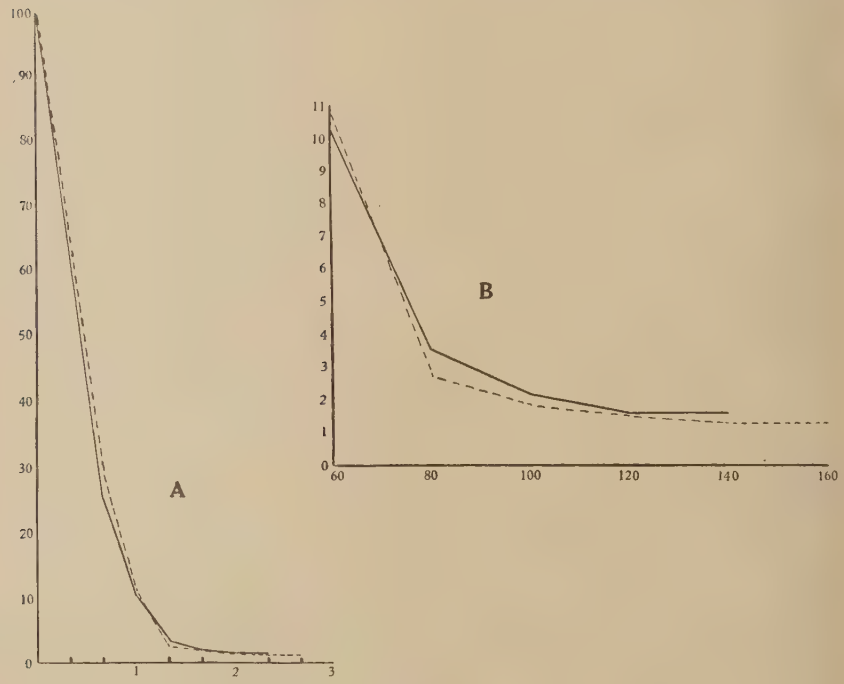


Fig. 3.

Rates of water loss from finely chopped leaves of Cupressus in January (dotted line) and Austrian Pine in November (continuous line). A—complete graph on same scale as Figs. 1 and 2. B—graphs from the 60-minute period on an increased scale. Ordinate × 5; abscissa × 3, and time plotted as minutes instead of hours.

protoplasmic pentosans really have any marked retarding effect on the rate of evaporation from the cell, it seemed that an examination of the drying rates of chopped leaves of those forms differing in a definite way in water-soluble pentosan content, should show that effect all the more clearly if the outer protective cuticle, hypodermis, and the like were, at least partly, removed by fine chopping, thus exposing the inner softer tissue to more direct dehydration. This was accordingly proceeded with. A characteristic result is shown in Fig. 3. Here are shown the drying rate for the Austrian Pine in November contrasted with that for Cupressus in January. The leaves were in each case rapidly and finely chopped with a sharp razor. During the chopping, of course,

there was some loss of water from sap exudation and evaporation, but comparison of many samples of the water content of quite similar material, some chopped and some unchopped, showed an average loss of about 1 per cent. of the total water. Such loss does not affect the final calculations sufficiently to be noticeable in the curves on their present scale. However, it should be stated that some of the leaves were not so readily reduced to the same degree of fineness as the others without spending such time upon them as would have meant an appreciable preliminary water loss. Lack of fineness in the grade of chopping resulted, occasionally, in slight slowing of the drying rates, but, even if all such differences were emphasized, the differences were of small amount, so that the graphs of Fig. 3 can be taken as a type of the data derived from measuring the rate of drying of chopped leaves. From an examination of these curves it is now clear, even if proof were indeed necessary at all, that the slow drying of the Austrian Pine is largely related to the protective outer layers of the leaf. The difference between Cupressus chopped and unchopped, as seen in Fig. 3 A and Fig. 1 respectively, is not nearly so great. It seems equally safe to conclude that the content of water-soluble pentosan has no effect on the rate of drying at 60° C. In spite of the great difference in such content between Cupressus in January and the Austrian Pine in November the drying rates of the two chopped are practically the same. Indeed, so close are they, that in Fig. 3 B, where the curves are redrawn from the 60-minute mark, a horizontal magnification in scale of three and a vertical one of five were necessary to permit of the actual points determined being properly distinguished.

It appears, then, that, either in chopped or unchopped leaves of Conifers, no relation exists between the rate of water loss, at least under the conditions of Rosa and Boswell, and the pentosan content. This agrees in a way with the old statement of Wisser (12), that no difference exists between the evaporation rates under uniform conditions of various plant saps and distilled water. Wisser experimented with saps from the principal ecological types. Spoehr (10), too, could find no difference between the drying rates of water and that of the mucilages of the Cacti. This opens also the question of the relationship of sap density and transpiration, and calls attention to the need of further work in Conifers along the lines of Bates (1) and Korstian (7) in their studies of sap densities of Conifers. Both claim that variations in sap density are the cause, rather than the result, of variations in transpiration, but as their results do not agree in many points, it is clear that so fundamental a point in Conifer physiology is still open.

But it is clear from the enormous disparity in the drying rates of chopped and unchopped Austrian Pine leaves what a great influence the mechanical structure of the leaf may have. It seems strange, with so obvious an interpretation of the main differences in the drying rates of hardened and unhardened cabbage, that Rosa and Boswell did not try some means of getting partly rid of this factor. They record the external changes in the leaves, which became tough, leathery, smaller, and covered with a waxy bloom in the hardened plants. There was an increase in the percentage of dry matter in the leaves—from, for example, 8·2 to 12·8—while the mesophyll cells, especially those of the palisade, were smaller, and more compact. In view of all these well-known xerophytic developments it is surprising that Boswell did not remove even the waxy bloom from the hardened cabbage leaves and test the effect of its removal on the rate of drying. This mechanical aspect of the drying rate crops up again in the study by Strasbaugh (11) of dormancy in the plum. Having shown that in hardy and semi-hardy plum varieties the twigs differ radically in their water relationships, he tested the drying rates over sulphuric acid of the hardy and

semi-hardy forms, finding a slightly greater drying rate in the semi-hardy forms. But he further discovered, however, that the twigs of the semi-hardy forms possessed from three to six times the number of lenticels to be found on the hardy types. Now the same idea of the forces of colloidal imbibition retaining moisture against the force of evaporation appears in an interesting recent paper by Edith Shreve (9) on the factors governing seasonal changes in transpiration of the desert plant *Encelia farinosa.* As it is hoped to deal more adequately with this later, it need only be said that the paper claims that difference in the transpiration rate in the wet and arid seasons is not due to leaf structure, but to an internal water-retaining and water-absorbing power. So much so that if O is the original water-content of the leaf, M its maximum water-absorbing power, E_d the evaporation from unit area of drying discs of the leaf, E_a the evaporating power of the air, then

$$\frac{E_d}{E_a} \times \frac{M}{O} = K.$$

If this be fully true it is an interesting result, but as it implies a factor in the dehydration similar to that postulated by Rosa and Boswell, an examination of the claim is very necessary.

A further point appears from the graphs drawn in Fig. 4. They show, on the same scale, the rates of water-loss of Austrian Pine leaves in late November (A), in October (B), and in early July (D); and of *Pinus montana* in September (C). The latter form is included to show that similarity between it and the Austrian Pine, to which allusion has earlier been made. The Austrian Pine leaves of July were the young leaves, fairly fresh from the bud and with the strong thickening of the cells, which is so characteristic of the older leaves of this species, not yet fully developed. Its rate of water-loss follows closely, in type, that of the other softer leaves examined, such as Tsuga and Cupressus. The exponential curve to correspond was not plotted, but this graph (D) obviously follows it fairly well. The other three curves, however, differ from it, but agree among themselves in the marked flattening below the middle. It must be remembered that no attempt was made to draw a smooth curve through the points obtained; they were joined directly by straight lines. Nor are these exceptional graphs; they are illustrations, taken more or less at random, of a regularly occurring phenomenon. Unfortunately, the interest of these was not at once realized, as they were not drawn till some considerable time after the drying; nor, indeed, is the method accurate enough or the data here available sufficient to permit a proper analysis of these drying rates. But they show that, in certain types of leaves, the question of water-loss, even from the dead leaf, is by no means a simple one. Miss Shreve's article calls attention to the chemical and physical complications while the leaf is still living. Apart from the whole stomatal question, however, the drying curve of the Austrian Pine shows that what may be called the living complications must be associated with a whole series of physical relations connected with the mechanical structure of the leaf. A glance at the graphs indicates that it is not merely a question of the slow rate of drying, but that the rate of evaporation is not the same during the process. The dehydration is discontinuous. The graphs seem to indicate that at the beginning and end of the process the rate of evaporation is proportional to the water content, but that, at a certain intermediate stage in the drying, it is practically constant over a definite period, bearing no relation to the water content. In view of this marked result in *Pinus montana* and *Pinus laricio,* even though these forms were so rapidly dried, it seems probable, pending further work, that corresponding changes

would be detectable in the other leaves if dried accurately and at a lower temperature; while a comparison between the drying rate of the young Austrian Pine leaves (D) and the older leaves (A, B) certainly seems to indicate that this discontinuity is to be related to the mechanical structure of the leaf. This discontinuity is very suggestive of the corresponding results obtained from the dehydration of such substances as wool, clay, sand, silt, and soil, published by Fischer (5, 6). An agreement also appears with the observations on the discontinuity of the hydration process by Davis and Eyre (3). Some of Fischer's remarks in this connection are of interest. Finding the dehydration

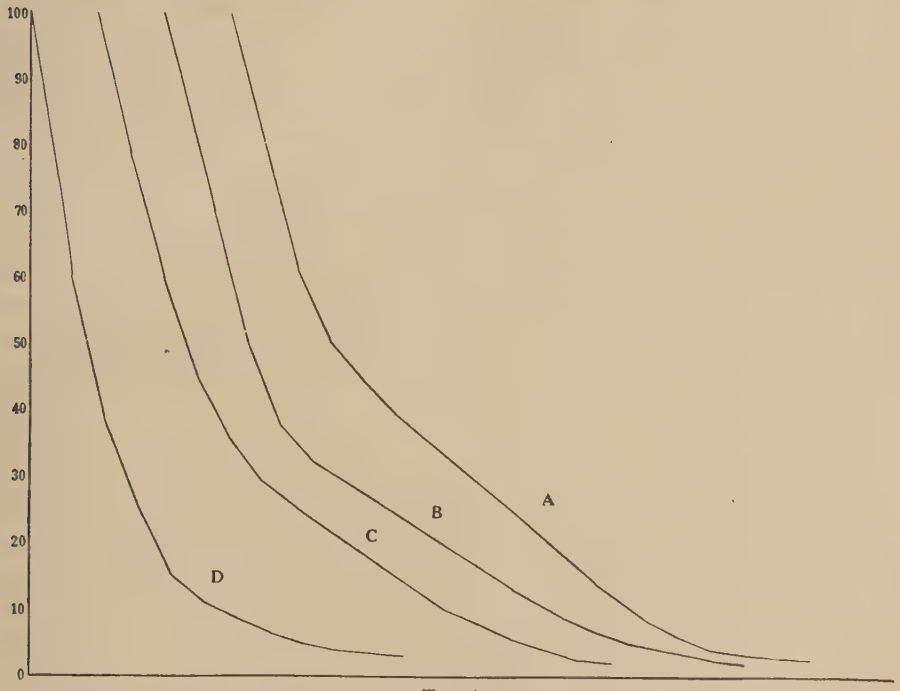


FIG. 4.

Rates of water loss from Austrian Pine for November A, October B, and July D; and from *Pinus montana* for September C: to show the marked flattening in the curve from the older leaves. Ordinates as usual, but abscissa not marked; the successive curves are moved to the right for a space corresponding to 40 minutes.

curves of wool, sand, and silt to be essentially the same, he concludes that a colloid, as such, has no characteristic rate-of-evaporation curve, and that it is only when other factors come into play, such as shrinkage in the case of clay, that certain peculiarities are impressed on the rate curves, peculiarities that have hitherto been almost universally ascribed to some peculiar property of the colloid state. He further explains the fact that certain parts of the rate-of-evaporation curve of clay are not linear but curved, as due to the different rates of evaporation of the interstitial water and the imbibitional or gel water.

The former evaporates at a rapidly diminishing rate, while the gel water evaporates at a steady rate till all the gel water is gone at about 7 per cent. water content. He further says, which especially interests us here, "that the water present in a gelatin gel and also the imbibitional water present in a swollen gelatin gel evaporate at a constant rate, which is comparable to that of free water in bulk of similar surface area: this constancy of rate of evaporation is maintained until the water content of the gelatine has been reduced to less than 100 per cent. (presumably 1 per cent. is meant), and the gelatin itself is as hard and as dry as horn" (5, p. 673). If these statements be substantiated it would seem that the whole of Rosa's theory is based upon an assumption which proves to be an incorrect one. That hydrophilous colloids have any power of retaining water against the tendency to either evaporation or ice-formation has apparently still to be proved. It seems a pity that Rosa did not inaugurate a series of physico-chemical experiments on the freezing of colloids, and thus not only fill a gap in colloidal chemistry, but at the same time provide himself with some basis for a theory which he propounded and supported with such skill.

It may safely be concluded on the data so far available (1) that there are no grounds for the claim of Rosa and Boswell that pentosan content affects the dehydration of plant tissues; (2) that any differences noted by them in the dehydration rates of cabbages subjected to various treatments may well be due to the mechanical differences in leaf structure, a factor which was not considered; and (3) that the dehydration of tissues is more complicated than would appear from their experiments, and obviously needs close and precise physical analysis. Of course, it is not meant to claim that the only factor in the evaporation of water from leaves is what we call the mechanical factor. But it is a factor, in some cases an important one, which was not properly considered by Rosa and Boswell.

The further data here presented also clearly show that Boswell's claim, that the rate of dehydration of tissues under uniform conditions is an index of hardiness, cannot be substantiated, at least as stated. Relationships between water and hardiness do exist, but have still to be analysed. It appears, too, that we must know more concerning the drying rates of hydrophilous colloids and the results of freezing them, considered from the physico-chemical aspect, before we can really visualize the mechanism of cold-resistance.

Summary.

1. Data are presented from the drying of Conifer leaves to show that the claim, that the rate of dehydration of certain plant tissues at 60° C. decreases with increasing content of water-soluble pentosan, cannot be substantiated.

2. It is also shown that mere rates of dehydration under uniform conditions may be no index of hardiness.

3. The dehydration of plant tissues is a complicated physical process which has still to be analysed.

The writers wish to extend their thanks to Mr. J. W. Besant, Keeper, Royal Botanic Gardens, Glasnevin, for allowing full use of so many of the Garden's trees; to Mr. A. C. Forbes, Director of the Forestry Branch, Department of Lands and Agriculture, for regular supplies of material from the Forestry Station at Avondale, Co. Wicklow, most of which supplies were carried up personally by him; and to Mrs. E. J. Doyle, for invaluable assistance in preparing the graphs.

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No. 25.

PHOTO-ELECTRIC MEASUREMENTS OF ILLUMINATION IN RELATION TO PLANT DISTRIBUTION. PART I.

By W. R. G. ATKINS, Sc.D., F.R.S.,

AND

H. H. POOLE, Sc.D.

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Introduction.

The study of plant distribution in relation to the illumination of the site has naturally been a subject of interest to botanists, but both the exact definition of the term "illumination," as applied, for instance, to a point just inside a wood, and the measurement of such illumination at any one time, have presented certain difficulties. Klugh (1925, 2) has reviewed and criticized previous work. His paper includes a lengthy bibliography, and introduces a new form of holder for photographic plates, which are exposed through various neutral tint and colour screens. This can be used both under water and in woods; it appears to be a serviceable apparatus, from which good approximate results may be obtained, the error claimed being about 5 per cent. Inasmuch, however, as the exposures made in the wood and outside it are not made simultaneously, a large error may arise owing to variations in the illumination in the open caused by almost imperceptible clouds. Against this error Klugh has guarded, as far as possible, by working only in settled weather. His results are recorded as percentages of the external illumination, and not only has the total intensity been measured, but, by the use of colour screens, records of the spectral character of the light have been obtained. Thus, in dense spruce woods at St. Andrew's, New Brunswick, on September 8th, 1924, with bright sun and a clear sky, Klugh found for red, λ 5900 A.U. and over, 0·005 per cent. of the external red; for green, λ 4900–5900 approx., 0·05 per cent.; and for blue, λ 4000–5000 approx., 0·12 per cent. It seems obvious from these results that almost the whole of the light must have passed through plant tissues or been reflected from them, since its spectral character has been changed so greatly. The apparatus used by us, and described below, was chiefly sensitive to blue light. The situations being more open than those examined by Klugh, a considerable amount of direct sky light was included. This, of course, has the effect of diluting, as it were, any change occasioned in the spectral character of the light, by transmission through, or reflection from, plant tissues. Since Kniep and Minder (1909), and Klugh (1925, 1), have adduced evidence that both red and blue light are effective in photosynthesis,[1] it seems established that our apparatus gives records which stand in a useful relation to the light energy available for photosynthesis, and so are a real aid towards the correlation of the light intensity of the habitat with plant distribution. We are, however, alive to the fact that a precise knowledge not

[1] The authors cited are in agreement that the green portion of the spectrum is of little service in photosynthesis.

only of the total intensity, as measured by the blue, but also of the intensities of the other portions of the spectrum, is desirable.

It should be mentioned that Klugh has severely criticized the comparison of light intensities by means of photographic P.O.P., as the law $E = It$, viz., the effect equals the intensity multiplied by the time, does not hold for photographic emulsions, nor can it be modified by the use of another constant "p" in the form $E = It^p$, for experiment has shown that "p" is not a constant.

In the present investigation use was made of a photo-electric apparatus, previously described by one of us (Poole, 1925) in these Proceedings, and used by us (1926) for measuring the illumination at various depths in the sea. The preliminary measurements and the testing of the apparatus were carried out on a pier in Cawsand Bay, Plymouth Sound. The idea underlying this work was to ascertain the depth to which light penetrates in amount sufficient to permit of appreciable photosynthetic activity. Above the pier is a wood, extending from Cawsand to Penlee Point, so the measurement of the illumination under and among the trees, and its relation to plant distribution, naturally presented itself as a subject for investigation.

The following short account of the method of working may serve to orient the reader desirous only of becoming acquainted with the results of botanical interest; it is followed by a consideration of the optical conditions involved, a description of the apparatus, the tabulated results, and their discussion.

Outline of Method Employed.

The apparatus consists of two photo-electric cells, suitably mounted, and connected to a potentiometer; the readings obtained are proportional to the intensities of the light incident on the windows of the photometers. The potentiometer and one photometer, which had only about a four-metre length of cable, were set up for use in the open; the second photometer, of which the sensitivity relative to the first had been ascertained, was connected by a fifty-metre cable. It was accordingly possible to place it at any desired spot in or near the wood within the limits of the cable length, and at any angle to the horizontal. Readings of the two photometers were then made in quick succession, the intervals being in many instances as short as twenty seconds. In each situation a series of such readings was made, commencing and ending with the open-site photometer. If the light was variable, possibly nine readings of the latter and eight of the shaded-site photometer were made; the values obtained were then averaged. Observations were also made of the illumination when the sun was unobscured, and when it was covered by thick cloud. The potentiometer readings were converted into metre candles, the photometer having been standardized in the laboratory, and the results were corrected for the effect of obliquity of the incident light on the losses due to reflection. The final result, in metre candles, is the illumination on a plane surface, horizontal or inclined as the case may be, at the site. This has also been stated as a percentage of the illumination in the open, as measured.

Optical Considerations.

It is necessary to define what is meant by the illumination at a given point. The problem is complicated by the fact that we have to deal with light travelling in many different directions; the angular distribution, moreover, varies greatly at different times. Thus, with a uniform cloudy sky, the angular distribution of the light may be approximately uniform over one hemisphere. On the other hand, if the sun is shining from a cloudless sky, the results

obtained indicate that 60–70 per cent. of the light may be direct sunlight, and may be treated as parallel rays, making a definite angle with the horizon.

Several definitions of illumination are possible. Thus, we may define the Vertical Illumination as the illumination in metre candles falling on a horizontal surface. This is what is generally measured by the photometers when certain small corrections have been applied for reflection losses at the front faces of the glass windows; it is denoted throughout by the letter *V*.

Another definition, which is very useful in photometric work in woods, is the illumination in metre candles on a surface set to catch the maximum amount of light. We will call this the Maximum Illumination, and denote it by the letter *M*. Evidently in the case of pure sunlight the photometer must be set perpendicular to the sun's rays. For actual sunlight, which contains some diffuse sky light, a setting rather nearer to the horizontal would receive more light.

A third definition is the one which was adopted in the 1925 paper, being there denoted by the letter *I*. It may be called the Total Illumination, and measures the total quantity of light regardless of angle, being defined as follows:—Let $id\Omega$ be the illumination in metre candles due to a small pencil of rays of solid angle $d\Omega$ on a small area placed perpendicular to the pencil. Then $I = \int_0^{2\pi} id\Omega$.

This measures the total illumination that is available for photosynthesis at a given point (neglecting, however, reflected light travelling upwards), although no single surface could be set to receive it all at once, except in the ideal case of pure sunlight. It is probably the best way of recording the photosynthetic activity of the light, and for that reason was adopted in the 1925 paper. Unfortunately it can only be found from the photometer reading by multiplying by a factor whose magnitude depends on the angular distribution of the light. This can be roughly estimated for open sites, knowing the altitude of the sun, from measurements of the relative proportions of sun and sky light. These factors for air (and water) were, in the 1925 paper, combined with the reflection loss factors, and in this way *I* was directly obtained from the photometer reading. As, however, the reflection loss correction is a small one, and only slightly affected by the angular distribution, it seems to be preferable to apply it separately, and thus obtain *V*, the quantity actually measured by the photometer. Accordingly in this paper results have been, as a rule, stated in terms of the Vertical Illumination, *V*, but for comparison values of the illumination recorded by an inclined photometer have also been given sometimes, these being particularly important at the border of a wood. It may be pointed out that the records made by Klugh and the workers whose papers have been reviewed by him appear to relate mainly to *V*, partly to *M*, and never to *I*. The factor for deducing the corresponding value of *I* for any given distribution of light may be obtained by interpolation between the pure sunlight and diffused sky light values given in Table 1, or the sunlight and diffuse light may be calculated separately. It is evident that, for a point lit by pure sunlight, $I = M = V \operatorname{cosec} \alpha$, where α is the altitude of the sun, for, in this case, to find *M* the photometer should be set perpendicular to the sun's rays, and, since *i* vanishes all over the hemisphere except in the small solid angle subtended by the sun's disc, $\int_0^{2\pi} id\Omega$ reduces to *M* simply.

TABLE 1.

Pure sunlight	α	$\operatorname{cosec}\alpha = \frac{I}{V}$	f	α	$\operatorname{cosec}\alpha = \frac{I}{V}$	f
,, ,,	5°	11·47	2·50	50°	1·305	1·01
,, ,,	10°	5·76	1·56	55°	1·22	1·005
,, ,,	15°	3·86	1·295	60°	1·155	1·005
,, ,,	20°	2·92	1·16	65°	1·105	1·00
,, ,,	25°	2·37	1·095	70°	1·065	1·00
,, ,,	30°	2·00	1·06	75°	1·035	1·00
,, ,,	35°	1·745	1·03	80°	1·015	1·00
,, ,,	40°	1·555	1·02	85°	1·005	1·00
,, ,,	45°	1·415	1·015	90°	1·000	1·00
Diffused light	30°	2·00	1·06	—	—	—

For perfectly diffused light, on the other hand, $M = V$, since the horizontal position catches the maximum light, and, as i is uniform over the hemisphere, $I = 2\pi i$. It may be shown by integration that, under these conditions, $V = \pi i$. So we have for perfectly diffused light $I = 2M = 2V$.

Actual unshaded daylight may be represented as a mixture of pure sunlight and perfectly diffused sky light. This is obviously only a rough approximation, as the sky is seldom uniformly bright all over, but it is the best that is practicable. An estimate of the relative proportions of sunlight and sky light can be made by taking readings of the standard photometer when freely exposed, and when shaded from direct sunlight by the interposition of a small object at a distance of several feet; the ratio we term β. Very similar results are given when observations are taken before and during the obscuring of the sun by a small cloud.

In order to find the illumination from the photometer reading we may proceed as follows. If I_s and I_d are the illuminations (whether total or vertical) due to pure sunlight and to diffuse light, respectively, and G and G' are the unshaded and shaded readings, then calling the factors for converting readings into illuminations x, we have since $\beta = \frac{G}{G'}$

$$I_s = x_1(G - G') = \left(x_1 - \frac{x_1}{\beta}\right)G$$

$$I_d = x_2 G' = \frac{x_2 G}{\beta}.$$

Therefore the total illumination

$$I_s + I_d = \left[x_1 + \frac{x_2 - x_1}{\beta}\right]G,$$

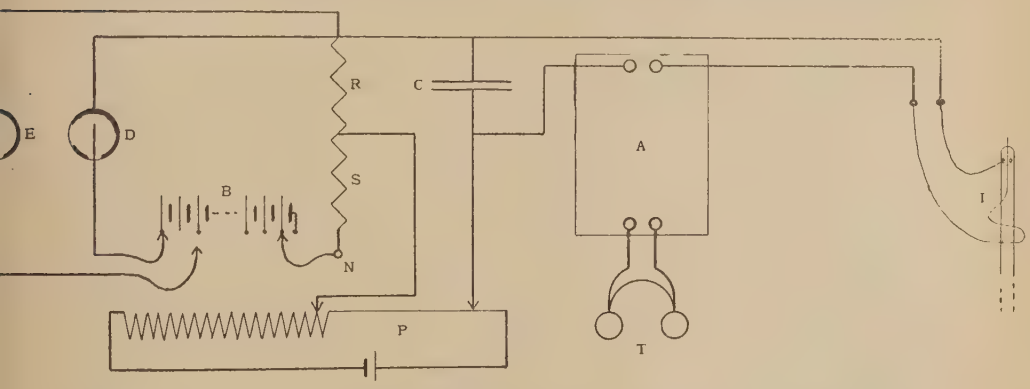
and so the corresponding factor for the total illumination is $x_1 + \frac{x_2 - x_1}{\beta}$, x_1 and x_2 being the values for pure sunlight (of the correct altitude) and diffused light, respectively.

This method of interpolation is used both for obtaining the ratio of the total to the vertical illumination, and for the estimation of the appropriate value of the reflection-loss factor "f," which, as explained below, must be applied to the photometer reading in order to find the vertical illumination.

For convenience in working a table was constructed, with the help of the Nautical Almanack, showing the altitude of the sun at each hour before and after local astronomical noon, which is approximately mid-way between the time of sunrise and sunset. The times recorded are Greenwich Mean Time. During the period, and at the hours, of work the sun's altitude was between 35° and 45°, so that the correction f was at all times small. The accurate knowledge of altitude is, however, of importance when calculating the total intensity I, from values of V, since, for pure sunlight, $I = V \operatorname{cosec} a$.

Method of Measuring the Photo-Electric Current.

The apparatus, as already mentioned, was devised for work at sea, which necessitated the substitution of a telephone for a galvanometer as a null-point indicator. For details as to its construction, reference must be made to the previous paper, from which the figure is reproduced to show the general scheme of the electrical connections. All subsidiary circuits, such as the potentiometer standard cell, the amplifier circuits, &c., have been omitted from the diagram for the sake of simplicity. B denotes the high tension battery, of



the usual wireless receiver type, which provides the anode potential for whichever of the photo-electric cells, D or E, is plugged into it. The current, which passes through the cell when exposed to light, also passes through a "Zenite" wire resistance, R, of 51,300 ohms, and a similar safety resistance, S, of about 19,000 ohms. The potential difference between the ends of R is measured by means of a potentiometer, P. Telephones, T, are used to detect any current due to lack of balance, this current being rendered intermittent by means of a special form of interrupter, I. The sensitivity is increased to a suitable extent by making use of a two-stage Marconi note magnifier, A, the amplification being controlled by varying the filament currents of the valves. The condenser, C, also increases the sensitivity. Guard ring wires, to prevent errors due to surface leakage, are connected to the negative terminal, N.

The interrupter, which is worked by an electric buzzer, forms an independent unit, which must be kept 5 or 6 feet away from the other circuits to prevent induction effects. The rest of the apparatus, with the exception of the photometers and their leads, and, of course, the head telephones, is rigidly mounted in a strong wooden box, about 60 cm. × 60 cm., and 30 cm. in height. This was built to stand use at sea in rough weather; it is a heavy load for one

man, and is a trying burden for two men up hill, and over rough ground; accordingly, for ecological studies, it would be desirable to assemble the apparatus in a more portable form, and this will, it is hoped, be done. In specially damp weather or late in the evening, when dew was being deposited on the insulating surfaces, trouble was experienced from "dark currents," *i.e.*, leakage currents, which prevent the potentiometer from reading zero when the photometer is screened from light. Provided these are small, say, 1–5 scale divisions, and fairly steady, it is easy to allow for them; their magnitude should, however, be checked at frequent intervals or their absence proved during each set of determinations. During the course of the measurements recorded in this paper, dark currents were never a source of trouble.

Photometers and Photo-Electric Cells.

Two photometers are employed. One, termed the air photometer in the other two communications, has been referred to above as the short-cable photometer, and is denoted throughout this paper by the letter *G*. It consists of a light-tight wooden box, with a sheet-metal cover, in the centre of which is a circular aperture 5 cm. in diameter, closed by a thin glass window, which is fixed to the under side of the cover with waterproof cement. The under side of this window is coated with the special matt varnish used for obscuring electric lamps. In contact with this surface is an opaque screen of black card, with a central aperture 2·4 cm. in diameter, forming the pupil of the photometer. This method of mounting ensures that the pupil receives light from almost a complete hemisphere, the screening due to the thin sheet-metal surrounding the window being negligible.

A vacuum photo-electric cell, made by the General Electric Company, is mounted inside the box, so that its pupil is almost in contact with the diffusing surface at the centre of the window.

The photometer is mounted on gimbals, an old compass mounting being used; this was originally adopted for use at sea, so that the diffusing surface might remain nearly horizontal, but its use on shore was found to be a great advantage, as it saved time and trouble in adjusting to the horizontal position. The gimbals could, however, be discarded for portability, and a spirit level used in adjusting. The three cables used to connect the anode, guard-ring, and kathode of the photo-electric cell, respectively, were four to five metres in length, and about 0·5 cm. in diameter. They have lasted well over the two seasons they have been in use.

Cables with much thicker rubber insulation, about 1 cm. in over-all diameter, were used with the second, or long-cable photometer (*H*), to guard against the possibility of damage, which might occur in taking the cables over rough ground with gorse in the open, and blackberry, hawthorn, and rose bushes in hedges and woods. No such trouble, however, was experienced, and it would possibly be permissible to use lighter cables; this would be an advantage on the score of portability. The three lengths of cable were wound on a drum, which had projecting X-shaped sides for ease in handling.

The long-cable photometer was similar in construction to the standard, or short-cable photometer, save that the potassium vacuum cell used was slightly larger, and the window was not coated with matt varnish; instead, frosted glass was used, the diffusing properties of which are not as effective. This would probably introduce serious errors with low-angle illumination, such as direct evening sunlight. The photometer was, however, intended for use mainly in shaded positions, and the matt varnish reduces the intensity of the light so greatly that, were it to have been applied to the window, the photometer would not have been sufficiently sensitive.

Standardization of the Photometers.

It has been shown by the Research Staff of the General Electric Company (1924) that the sensitivity of a vacuum photo-electric cell, such as is used in this photometer, is constant, and the current through the cell is very nearly proportional to the illumination. The short-cable photometer was, accordingly, standardized in the Physical Laboratory, Trinity College, Dublin, and was used to standardize the long-cable photometer at Cawsand. Both cells had potassium as the active element. Shinomiya (1919) and Miss Seiler (1920) have shown that the maximum sensitivity of these cells is in the neighbourhood of 4,500 A.U., *i.e.*, in the blue part of the spectrum. The cells, accordingly, measure chiefly blue light, and in standardizing it is best to use a source as similar as possible to daylight. For this reason, an open carbon arc was used to standardize the photometer, although it is in other respects somewhat unsatisfactory, owing to its irregularity. During the 1924 experiments a ground-glass window was used, and the sensitivity had been found to be 195 metre candles per potentiometer scale division [10^{-4} volt].

As, however, doubt was felt as to the diffusing efficiency of the ground-glass surface at large obliquities, the window was subsequently replaced by the matt-varnished one, as previously mentioned, and the photometer was restandardized.

A 36-watt 12-volt gas-filled lamp was compared by means of a flicker photometer, first with a 16-C.P. standard carbon filament lamp, working at 4·05 watts per candle, and then with an open carbon arc, carrying a current which was kept as nearly as possible at 10·0 amperes by hand regulation, the voltage across the arc being about 59·5. The mean of four readings gave the candle power of the arc in a definite direction as 953, the maximum and minimum values being 1070 and 856.

Five readings of the photometer were then taken with the arc at a distance of 50 cm., and three with the arc at 30 cm. from the window, the direction of illumination relative to the arc being the same as before. The readings showed considerable variations, due to fluctuations in the arc; but the means of the two sets, 12·7 and 34·5 scale divisions, respectively, agree within 2 per cent. with the inverse square law. A sensitive galvanometer was used as a detector, thus considerably increasing the accuracy of the setting. As the "Zenite" resistance fitted into the apparatus box was not available, a similar one was used, its resistance being 48,070 ohms. The anode potential was 118·5 volts. With the other "Zenite" resistance the sensitivity would be increased 1·067 times, while increasing the anode potential to 120 volts would raise it about 0·45 per cent. Making allowance for these corrections we find that, with an anode potential of 120 volts, one potentiometer scale division corresponds to 282 metre candles.

An alternative standardization, with a Mazda gas-filled "Daylight" lamp, *i.e.*, a lamp with a blue-tinted bulb, affording a light resembling daylight visually, gave 466 metre candles per scale division. This large difference may possibly be due to the deficiency of violet and ultra-violet in the light of the metal filament lamp. It seems probable that arc light resembles daylight much more closely than that of the "daylight" lamp as regards its effect on the photo-electric cell. Accordingly, the arc lamp standardization has been used throughout, although there is little doubt that, as daylight is bluer than arc light, its effect on the photometer is relatively greater; so that the results found for daylight illumination on this photo-electric scale are all higher than they would be on the visual scale.

TABLE 2.

Determination of the ratio of the sensitivity of the two photo-electric photometers, viz., of the cells when mounted for use, *G* being that with the short cable:—

Date	G.M.T.	*G*	*H*	*H*/*G*	Weather conditions
Sept. 11	11·17	213	1186	5·57	Sun out, good set.
„ 23	10·59	192	1439	7·49	Sun clear.
„ 14	1·30	190·4	1200	6·30	Sun out.
„ 23	10·54	184	1370	7·45	Sun clear.
„ 14	1·40	183·5	1122	6·11	Sun out.
„ 24	10·42	168	1163	6·92	Sun clear.
„ 14	10·6	152	1009	6·64	Sun out.
„ 7	12·40	78	485	6·22	Diffuse light, good set.
„ 11	2·3	55·2	347·2	6·29	Diffuse, mean of variable readings.
„ 14	1·40	54·8	384	7·01	Diffuse, shaded from sun.
„ 11	2·18	45·8	313	6·83	Diffuse.
„ 24	10·38	42	318	7·33	Diffuse, dark clouds.
			Mean	6·68	All values.
			Mean	6·73	Diffuse, light values.
			Mean	6·64	Direct sunlight values.

As shown in Table 2, the mean of the values for the ratio of the sensitivity of the two photometers is the same, very closely, irrespective of whether they were obtained in sunlight or in wholly diffuse light, and irrespective of the actual values of the readings. It seems clear, then, that the two cells give comparable readings when used as intended. Making use of the mean value for the ratio of the readings of the two photometers, *G* and *H*, viz., $H/G = 6{\cdot}68$, it is seen that with *H* one potentiometer division corresponds to 42·2 metre candles with anode potential 120 volts.

It is of interest to determine to what extent the matt surface and the ground-glass windows diminished the light incident on the cells *G* and *H* respectively. A comparison was, accordingly, made. In clear sunlight, the sun's altitude being 39½° and the anode potential 118·2 v., *G* gave a reading 156 on the scale, and 1518 after the lid of the box had been removed; the ratio is accordingly 9·73 and the transmission factor of the window 0·103 under these conditions. In sunlight, the long-cable photometer, *H*, gave a reading 841 with 118·2 v., and 614 with 18·0 v., the readings being off the scale when the cover was removed. A little later, however, with the sun obscured by cloud and anode potential of only 6·2 v., photometer *H* gave a reading 102, increased to 385 when the cover was removed; the ratio is thus 3·78, and the transmission factor 0·265. The readings given here are, as usual, the means of their respective series. From these results it is seen that the matt surface reduces the reading about tenfold, and the ground-glass less than fourfold. The retention of the latter for use in the long-cable photometer, accordingly, seems justifiable. An attempt is being made, by the use of a conical reflector, to construct a photometer which will give a satisfactory measure of oblique light without the large loss caused by the matt varnish.

The irregularities in the determinations of the ratio H/G are possibly due to the H photometer, which was not on gimbals, not having been set accurately horizontal. The mean of all values, 6·68, was taken in determining the constant for H, viz., 42·2 m.c. per scale division, and this value has been used in all the calculations of illumination in the shade recorded in this paper. On examining Table 1, however, it has since been noted that the three observations made on September 23rd and 24th, in full sunlight, gave values 7·49, 7·45, and 6·92, mean 7·29; and one on 24th, in diffuse light, gave 7·33; mean of all four 7·40. In these determinations care was taken to set H accurately by means of a spirit level. Owing to attention being focussed on possible differences between "sun" and "shade" values, this point was previously overlooked. It will be tested afresh when work resumed; but if the factor $H/G = 7{\cdot}40$ is more accurate than 6·68, all the shade illuminations, as recorded in metre candles, will require to be reduced 10·8 per cent., namely, the constant becomes 38·1 m.c. per scale division rather than 42·2 m.c., at anode potential 120 v.

Recent laboratory work with another photo-electric cell has shown that not only does the variation of sensitivity with obliquity for the bare cell deviate somewhat from the cosine law, but also the sensitivity for a given obliquity may vary with the azimuth of the illumination. If this effect occurs in the cells used in the H and G photometers it would be greatly reduced by the ground-glass in photometer H, and, probably, rendered very small indeed by the matt varnish window of photometer G, whose sensitivity to oblique light was found to agree well with the cosine law. It is possible, however, that the variations found for the ratio H/G are due to variations in the sensitivity of H to oblique light of varying azimuths.

Reflection Losses at Photometer Windows.

Since it is obviously necessary to protect the diffusing surfaces, these form the inner faces of the glass windows. We must, accordingly, correct for the variation with obliquity of the reflection loss at the front surface of the window. This may be done by means of Fresnel's formulæ. Assuming the refractive index of the window-glass to be 1·51, the loss at normal incidence in air is about 4·1 per cent. For oblique angles, the loss increases, very slowly at first, and more and more rapidly with increasing obliquity. The G photometer was standardized at normal incidence, as described above; hence the factor, "f," by which the reading must be multiplied to correct for reflection losses, reduces to unity in this case. Its values for other altitudes of illumination are given in Table 1.

The Illumination due to Direct Sunlight and to Diffuse Sky Light and its Variation.

Walsh (1923) has summarized our knowledge of daylight and its variations as determined from the illumination of test cards exposed horizontally in an open field. The measurements were made at the National Physical Laboratory, Teddington, between March and December, 1914. Direct sunlight was always shielded from the test cards. The work of Paterson and Walsh (1915) on the illumination of rooms has a direct bearing on the lighting inside a wood. They showed that on a dull day, with a grey sky, the illumination at a point inside a building bears a very nearly constant ratio to the illumination at a point outside. This ratio, expressed as a percentage, has been termed the daylight factor at the point, and is taken as giving some indication of the illumination when the outside illumination is known. They found that the sunrise and

sunset illuminations do not vary with the time of the year, but only with the meteorological conditions, and may be assumed to lie, generally, between 100 and 500 metre candles; 250 m.c. may be taken as a fair average value. Curves are given to illustrate the illumination on average days in June, September, and December. The mid-day illumination, converted to metre candles, is shown in Table 3, quoted from their results given as foot candles.*

TABLE 3.

MONTH.	Mid-day vertical Illumination in metre candles. Diffused light only.		
1914.	Highest.	Lowest.	Mean.
April ...	57,460	7,640	34,430
May ...	58,430	11,300	34,430
June ...	55,520	26,040	44,130
July ...	Sufficient observations not available.		
August ...			
September ...	32,600	9,360	12,700
October ...	28,080	1,640	13,990
November ...	26,910	1,400	11,840
December ...	12,480	3,870	7,750

The values given in Table 3 may be compared with those shown in Tables 4–9, since the latter include some in which direct sunlight was cut off by a small cloud. The results are in good general agreement. It is of interest to note that the mean value for the illumination (V) in June is more than five times as great as that in December. Were the values of β known for the various months and weather conditions, it would be possible to apply these results directly in a work on plant growth, taking into account the average amount of sunshine in the seasons. The writers (1926) carried out determinations of β at sea in the autumn of 1925, the entire hemisphere of sky being unobstructed, save for the small effect of the funnel, spars, and rigging. When the sky is overcast the ratio is, of course, 1·0. On September 2nd, between 9.35 and 10.32 a.m., β was 3·5, save when a light cloud over the sun reduced it to 1·5. At 10.37 a light cloud over the sun lowered β to 2·5. From 10.42 to 11.8 β was 3, and the general illumination was less. Sometimes the approach of a cloud towards the sun increases the general illumination by 7 per cent. or more, and so reduces β. In general, β is lowered by reflection from clouds as the diffuse light is increased. On September 3rd, at sea, values of β were close to 3 from 9.30 to 10.30, but from 12.30 to 2 p.m. they ranged from 1·0 for "dull" to 1·5–2·0 for "weak sun." Again, on October 1st, "weak sun," with much haze, gave $\beta = 1{\cdot}5$. Other values will be found in Tables 4–9; but some of the records, those for bases Nos. 3 and 4, were shaded from sky light by a wood on the west; they accordingly give fictitiously high values of β, which rise even higher as the sun approaches the edge of the wood.

The position of the potentiometer and photometer G has been, and will be,

* One foot candle = 10·763 metre candles.

referred to as the base; from each base an area, denoted by the same number, was examined; in each area the positions chosen for the placing of photometer H are termed the sites, and are denoted by numerals and letters—as 1 A, 1 B, &c. The description of the vegetation at any site has been limited to its immediate vicinity, as defined by similarity of conditions of illumination.

Illumination in Shaded Situations.

As pointed out in the previous section, a site exposed to full sunlight may nevertheless receive less than the maximum amount of light, since it may be screened from a portion of the diffuse light. Such positions, near the edges of woods and close to walls or banks, often show well-marked differences in their flora as compared with sites quite unshaded. When the sun is hidden, they obviously have a daylight factor, which may deviate largely from 100 per cent. The position cannot, however, be described correctly in terms of the daylight factor alone, since it receives sunlight also. As a rule, such positions are only exposed to the sun for a portion of the day. Suppose a site receives 75 per cent. of the diffuse light, viz., has a daylight factor 75, and goes into shade at noon, β being 3 in the open, viz., total light 3, diffuse light 1, sunlight 2. The site will, therefore, receive, up to noon, a total illumination $2 + \frac{3}{4}$, and β will be, for it, $\frac{2{\cdot}75}{0{\cdot}75} = 3{\cdot}7$ (nearly). After noon the illumination will be $\frac{3}{4}$; so, for the whole day, the score for the site will be $2{\cdot}75 + 0{\cdot}75 = 3{\cdot}5$ out of a possible 6, assuming continuous sunshine throughout the day. This also assumes that $\beta = 3$ remains constant for the day. On this hypothesis, we may say that the site has a total light factor of approximately 58 per cent. As, however, β would decrease towards morning and evening, the true value for the site must lie between the daylight factor, 75 per cent., and this 58 per cent. Since the meteorological returns for England, S.W., show an average of 36 per cent. of the total possible sunshine during the leafy months, May–October, 1923, inclusive, the values may be weighted, and the site rated approximately as $\frac{58 \times 1 + 75 \times 2}{3} = 70$ per cent. almost. For the whole year the average of the possible sunshine is 32 per cent.; so it is clear that in situations exposed only to morning or afternoon sun the daylight factor is more important throughout the year than is the total light factor. Moreover, in a wood, as may be seen from inspection of the tables which follow, a change in the external direct lighting, such as the obscuring of the sun by a light cloud, may have but little effect unless the position receives also shafts of sunlight.

Tables 4–9, accordingly, contain, in addition to the intensity measurements and data relevant to them, the ratio V_s, for the shaded site to V_0, for the base, which, when $\beta = 1$ and the base is unshaded, is the daylight factor When the sun is shining the diffuse light at the base is $\frac{V_0}{\beta}$, and the daylight factor for the shaded site becomes $\frac{V_s \beta}{V_0}$, the values of which are tabulated. This assumes that in the wood the illumination depends only on the diffuse light. The agreement shown in Tables 4 and 5 between values for this factor determined when the sun was out and when it was obscured indicates the correctness of the assumption for these particular sites, though such good agreement is not always shown, as, for example, in Table 8, site C, where the value 0·40 per cent. must have been about half due to light reflected from shafts of direct sunlight, such as were observed all round in that part of the wood.

Bases 3 and 4 gave fictitiously high values of β, since they were somewhat shaded by the neighbouring wood. In this case the interpretation of the results is complicated by the fact that neither V_0 nor β, as found at the base, has its true unshaded value. We can make an approximate allowance for this effect if we assume, from past experience, a value β' for the given conditions in the open. For, since the direct sunlight must be $\frac{\beta - 1}{\beta} \cdot V_0$ and also $\frac{\beta' - 1}{\beta'} V'_0$, where V_0' is the value that would be obtained in an unshaded position, we see that $\frac{V_0}{\beta} = \frac{\beta' - 1}{\beta - 1} \cdot \frac{V_0'}{\beta'}$. Thus the daylight factor for the base is $\frac{\beta' - 1}{\beta - 1}$. We can thus obtain an estimate of this factor, δ_0, from sunshine readings at the base. Then the daylight factor for a shaded site would be $\delta = \frac{V_s \beta \delta_0}{V_0}$. It would be better, though not always practicable, to measure δ_0 directly by taking readings with the *H* photometer at the full cable length from the base directly away from the wood. In the absence of such readings values for the daylight factors of bases 3 and 4 have been deduced on the assumption that for clear sunlight $\beta' = 3$.

Tables 7–9 include an extra column for the corrected daylight factor $\frac{V_s \beta \delta_0}{V_0}$. In tabulating the daylight factors of the sites, in which various species were found growing, the corrected factor has been used for data from Tables 7–9, as giving results more truly comparable with those of other sites.

At the outset, determinations were made of V_s. Tables 6, 8, and 9 also record measurements made with the photometer sloping at various angles, and towards different points of the compass, in an endeavour to obtain the maximum illumination in shade, M_s. Some values entered under V_s are, therefore, in reality values of M_s, and others are intermediate between the two, or even less than V_s; for convenience, the heading V_s has been retained, the position of the photometer being shown in a separate column. The increased illuminations—up to M_s—indicated by sloping the photometer, must be capable of supporting a more intense photosynthetic activity, and are best tabulated as daylight percentages. They are accordingly shown as such in the tables. In no case was the photometer sloped towards the sun.

It must be remembered, however, that, owing to the presence of other plants all round, seedlings are mostly dependent upon values of V_s rather than M_s so that unless a seedling becomes well established when the trees are bare a low value for V_s may control its further growth, even where the value of M_s appears favourable.

General description of the locality studied.

The Cawsand-Penlee wood lies on a steeply sloping hill, which faces east and constitutes the outer boundary, to the west, of Plymouth Sound. It is accordingly sheltered from the force of the south-westerly gales. The region between Cawsand and Penlee Point is on the geological formation known as the Dartmouth Slates, which is the lowest portion of the Lower Old Red Sandstone (Devonian Series) found locally. The wood is a mixed plantation with oak, beech, ash, sycamore, horse-chestnut, sweet or edible chestnut, evergreen oak, hawthorn and rhododendron. Young trees regenerate freely in it. About ten metres from the edge of the wood the character of the vegetation becomes more or less uniform and adapted to the characteristics of the wood, ground

ivy (*Hedera helix*), woodbine (*Lonicera periclymenum*), and straggling undersized blackberry (*Rubus fruticosus*) predominating. The areas examined were four in number, No. 1 being a gully just above sea-level and facing eastwards on Pier Cellars Harbour. This gully has almost vertical sides varying from about six to sixteen metres in height. Save in the early morning the southern face is therefore in shade. Areas Nos. 2 and 3 were more or less traverses from open ground into the wood to the full extent of the cable. No. 4 was around the outskirts of the wood. They were chosen according to the type of vegetation, as illustrating the shading due to different species of trees. In view of the well established importance of hydrogen ion concentration as a factor influencing plant distribution, soil samples were taken for each site at a depth of 1–5 cm.; the *pH* value of the soil extracts was determined as previously described (Atkins, 1922, 1924). It has been pointed out by Sager (1923) that there is a progressive increase in soil acidity according as a wood is entered and light becomes reduced in intensity. The few data provided by this investigation are quite in accord with Sager's results. In the gully area No. 1 the values were *pH* 7·0–7·2 in the open to *pH* 6·2–6·5 in the shade. In the wood the reaction varied from *pH* 6·0–6·2 near the edge to *pH* 5·6–5·7 further in.

Each site was worked with the potentiometer outfit, and *G* photometer at one fixed base; thus, for area No. 1 the base was on the pier and served to work all the sites in the gully. For convenience of reference these will be denoted Nos. 1 A–1 F, and similarly for the other sites. Values of β were always determined with the standard photometer in the open near the apparatus box. For convenience of comparison the *pH* values of the soil and the daylight factors (δ), as tabulated in Tables 4–9, are quoted with the list of plants found at each site.

The flora and light intensities in the various areas and sites.

Area No. 1, as in Table 4.

Site A.—Near east, viz., open, end of gully, supporting mixed grasses, *Plantago lanceolata, Angelica sylvestris, Rubus fruticosus; pH* 7·0. This receives sunlight in morning, but is shaded on the west; daylight factor, $\delta = 42$. The values of δ quoted here are calculated from V_s, horizontal setting.

Site A′.—In middle of gully, further in than *A*, mixed grasses, *R. fruticosus, Eupatorium cannabinum, Arctium lappa*; receives some morning sunlight; $\delta = 34$.

Site B.—Under north cliff of gully, beneath bushes of *Prunus communis* and *Crataegus oxyacantha*, dead leaves not grass, *Urtica dioica, R. fruticosus, Solanum dulcamara, Galium mollugo, pH* 7·2; receives sunlight like A; $\delta = 16·5$.

Site C.—In middle of gully, further in than A′. *Urtica dioica* dominant and beyond in a large wet patch, *Nasturtium officinale*; *pH* 6·6; $\delta = 37$.

Site D.—Close to S. bank of gully, under leaves of a very large specimen of *Pteris aquilina*, no outlook; well developed *Scolopendrium vulgare*, a very small *Hedera helix*, *U. dioica* one small plant, moss; moist soil, *pH* 6·5; $\delta = 1·9$.

Site E.—About 20 cm. to E. of previous position, under edge of *P. aquilina*, one tall *U. dioica*, no grass, carpet of *Chrysosplenium oppositifolium* just beginning; *pH* 6·2; $\delta = 1·9$.

Site F.—About 1·6 m. to E. of previous position at outer edge of carpet of *C. oppositifolium*, just next carpet of *N. officinale*, *U. dioica* a few plants, *Lychnis diurna* one plant; moist ground, *pH* 6·8, $\delta = 16$.

The details concerning the illumination of the foregoing sites are given in Table 4.

TABLE 4.

Sept. 7th, 1925.—Area No. 1. Base, on concrete pier, enclosing Pier Cellars boat harbour, Cawsand; cable and one photometer taken across harbour into steep-sided gully facing east. β determined in open at base. Wind N.W.

Area No. 1. Site.	Weather conditions.	G.M.T.	β	Illumination, metre candles. In open. V_0	In shade. V_s	Percentages Daylight factor. $\frac{V_s}{V_0}$	$\frac{V_s\beta}{V_0} = \delta$	pH
A	Sun through light clouds ...	1.2	2·4*	49,100	18,700†	38	—	7·0
„	Dark clouds over sun ...	1.9	1·0	20,900	8,120	42	42	7·0
A′	Do.	1.34	1·0	16,600	5,620	34	34	—
B	Do. ...	1.20	1·0	23,400	3,870	16·5	16·5	7·2
C	Do. ...	1.54	1·0	22,300	8,230	37	37	6·6
„	Sun clear between light clouds.	1.59	—	73,600	12,900	17·5	42‡	6·6
D	Dark clouds over sun ...	2.15	1·0	20,300	390	1·9	1·9	6·5
E	Sun out, clearer sky ...	2.38	—	58,700	540	0·92	1·9§	6·2
„	Do. ...	2.45	3·4	50,800	—	—	—	6·2
F	Sun out, β relative to 3.12 obs.	3.6	2·5	52,200	2,800	5·5	16‖	6·8
„	Base No. 1 now just in shadow of wall, sun behind light clouds.	3.12	1·0	20,600	3,500	17	17	6·8

* β relative to 1·9 shade value. † Received some sunlight.
‡ Calculated from $\beta = 2·4$, comparison with previous value 37 per cent. shows that β must have been slightly over 2·1. § Using $\beta = 2·1$. ‖ Using β = mean of 3·4 & 2·5.

Area No. 2, as in Tables 5 and 6.

Site A.—On path just below bank outside the wood, receives full sunlight up to about 11 a.m., but is shaded by wood. Mixed grasses and *R. fruticosus*, 61–52 per cent. of total daylight when examined, including some sunlight.

Site B.—Under first tree, *Fagus sylvatica* at edge of wood, good N.E. aspect, *Quercus robur* scrub, *Acer pseudo-platanus*, young, *Castanea vulgaris*, young, *P. aquilina*, *R. fruticosus*, *Rosa arvensis*, *Bromus asper*, a little *H. helix*, *Geranium Robertianum*, *Lonicera periclymenum*, and a little nearer the light, *Tamus communis*, twining on *P. aquilina*; *Scilla nutans* frequent in spring; dry soil, *pH* 6·2; δ = 16–18. About a metre nearer the light *E. cannabinum*, *Galium mollugo*, and *Dactylis glomerata*. These three may, therefore, require more light than δ = 18, *E. cannabinum* occurs at 1 A′ where δ = 34, though at 1 B *G. mollugo* occurs with δ = 16·5.

Site C.—Under second tree (*F. sylvatica*) in wood, 10 m. further in, much *H. helix* straggling on ground, also *L. periclymenum* and stunted straggling *R. fruticosus*, some *P. aquilina*, *Fraxinus excelsior*, young, *Castanea vulgaris*,

young, *A. pseudo-platanus,* young, *Q. robur,* young, *Rosa arvensis, Rubia peregrina, Lysimachia nemorum,* no grass; *S. nutans,* frequent in spring; dry soil, *pH* 5·7; $\delta = 2{\cdot}9$.

Site D.—About 11 m. further in under young *F. sylvatica,* over-arched by other trees, *H. helix* straggling dominant, a little stunted *R. fruticosus* and *L. periclymenum* straggling, one plant *Primula veris*; dry soil, *pH* 5·8; $\delta = 1{\cdot}8$. *S. nutans* frequent in spring, *Mercurialis perennis* absent.

The details concerning the illumination at the foregoing sites of area No. 2 are recorded in Tables 5 and 6.

TABLE 5.

Sept. 10th. 1925.—Area No. 2. Base in open, near edge of cliff, 120 metres east of Cawsand wood. All values of β refer to base. Photometers horizontal. Wind N.W.

Area No. 2. Site.	Weather conditions.	G.M.T.	β	Illumination, metre candles. In open. V_0	In shade. V_s	Percentages Daylight factor. $\frac{V}{V_0}$	$\frac{V_s\beta}{V_0}$	*pH*
A	Sun through light clouds	11.13	—	33,800	20,600	61*	—	—
„	Sun out, clear sky ...	11.19	—	74,700	38,900	52*	—	—
„	Do. ...	11.53	3·1	—	—	—	—	—
B	Sun out, light fleecy clouds	11.55	—	81,500	4,770	5·8	18·1†	6·2
„	Clouds over sun ...	11.59	1·0	25,400	4,630	18·3	18·3	6·2
„	Sun out, blue sky, light clouds.	12.2	—	76,700	5,310	6·9	20·7‡	6·2
	11.55 with 11.59 ...	—	3·2	—	—	—	—	—
	11.59 with 12.2 ...	—	3·0	—	—	—	—	—
C	Cloud over sun ...	12.42	1·0	25,400	730	2·9	2·9	5·7
„	Sun through light clouds	12.45	—	54,100	830	1·5	3·1§	5·7
	12.42 with 12.45 ...	—	2·1	—	—	—	—	—
D	Cloud over sun, position No. 2 now shaded by wood.	2.5	1·0	34,400	620	1·8	1·8	5·8

* Site 2 A got sunlight through topmost branches of wood at hour of experiment.
† From $\beta = 3{\cdot}1$. ‡ From $\beta = 3{\cdot}0$. § From $\beta = 2{\cdot}1$.

TABLE 6.

Sept. 23rd, 1925.—Area No. 2 (repeated). Base in open, near edge of cliff, 20 metres east of Cawsand wood. All values of β refer to base. Wind N.W. V_s is only, strictly speaking, the vertical illumination, when photometer is horizontal, but the symbol has been used also for inclined positions, for convenience in tabulation.

Area No. 2. Site.	Weather conditions.	G.M.T.	β	Illumination, metre candles. In open. V_0	In shade. V_s	Position of photometer in wood.	Percentages Daylight factor. $\frac{V_s}{V_0}$	$\frac{V_s\beta}{V_0}$	pH
B	Sun clear, clear blue sky, clouds to N.	11.28	—	50,800	2,270	Horizontal	4·5	16*	6·2
		11.32	—	51,000	5,360	Sloping 30° E.	10·5	37	6·2
„	Do. ...	11.35	—	50,800	6,190	„ 60° E.	12·2	43	6·2
„	Do. ...	11.37	—	50,500	6,030	„ 75° E.	12·0	42	6·2
C	Sun clear	11.42	—	51,900	970	Horizontal	1·9	6·5	5·7
„	Sun clear, increase in	11.44	—	57,000	1,510	Sloping 30°–40° N.	2·6	9·3	5·7
„	light from thin cloud	11.48	—	53,000	810	„ „ W.	1·4	4·8	5·7
„	near sun diffusing	11.52	—	54,100	1,480	„ „ E.	2·7	9·5	5·7
„	light.								
D	Sun clear	11.56	—	51,300	520	Horizontal	1·0	3·5	5·8
„	Do.	11.59	—	51,900	900	Sloping 30°–40° N.	1·7	6·0	5·8
„	Do. ...	12.3	—	53,300	450	„ „ N.E.	0·84	2·9	5·8
	Sun clear	12.14	3·5	53,000	—	——	—	—	—
	Sun clear, clouds bearing down in N.E. sky.	12.19	2·8	58,900	—	——	—	—	—
	Rain	12.22	—	—	—	——	—	—	—

* The value of β used is that for 12·14, viz., 3·5, throughout the column.

Area No. 3, as in Tables 7 and 8.

Site A.—Under large *Castanea vulgaris* which stands at edge of wood, on bare earth patch near bole of tree, *P. veris* next bole, some moss around, and further towards light were large fern, large *R. fruticosus*, and low bush of *Laurus nobilis*; dry soil, *pH* 6·0; $\delta = 1{\cdot}2$.

Site B.—Three metres further into wood than A, under boughs of the chestnut, on bare patch beside a few stunted plants of *Rumex obtusifolius* and tufts of *Bromus asper*. Near the site, *R. fruticosus, P. veris, F. excelsior,* young; dry soil, *pH* 6·0; $\delta = 1{\cdot}6$.

Site C.—About 15 metres further into wood, under tall *F. excelsior*, general shading from over-arching branches of *A. pseudo-platanus, F. sylvatica,* and *Q. robur,* on bare earth and dead leaves, straggling *H. helix,* stunted *R. fruticosus, Aspidium* sp., *Scolopendrium vulgare, Mercurialis perennis* (very plentiful in spring), *Circaea lutetiana,* in fruit, but with larger and thinner leaves than when growing in more open positions; young *F. sylvatica, Scilla nutans,* frequent all round in spring, also *P. veris,* further up; dry soil, *pH* 5·7; $\delta = 0{\cdot}8$.

Site D.—Between *C. vulgaris* of site A and another large tree of same species, *F. excelsior,* young, *A. pseudo-platanus,* young, on earth and leaves beside one plant of *R. obtusifolius*; dry soil, *pH* 6·1; $\delta = 0{\cdot}9$–$3{\cdot}2$.

Site E.—Under *Rhododendron,* sp., growing on inner side of and under the *Castanea* of site D, ground bare, save for moss and very small unidentified fern; ground not so dry, *pH* 5·6; $\delta < 0{\cdot}05$.

Site F.—About 25 cm. from site E, on dead leaves near edge of rhododendron, one small straggling branch of *H. helix*; $\delta < 0{\cdot}09$. Position was apparently better illuminated than E, but change in value of δ was occasioned by diminution in external illumination. It has been assumed that nothing lower than 0·5 scale division, or 20-metre candles, can be detected. The actual readings at sites E and F were zero.

The details concerning the illumination at the foregoing sites of area No. 3 are recorded in Tables 7 and 8.

TABLE 7.

Sept. 11th, 1925.—Area No. 3. Base in open, near edge of cliff, 19 metres east from rail at edge of Cawsand wood, further north than No. 2. All values of β refer to base. $\delta_0 = 77$ per cent., assuming $\beta = 3{\cdot}6$ (mean sunshine value). Wind N. W. Photometers horizontal.

Area No. 3.			Illumination, metre candles.			Percentages Daylight factor.			
				In open.	In shade.	Apparent.	Corrected.		
Site.	Weather conditions.	G.M.T.	β	V_0	V_s	$\frac{V_s}{V_0}$	$\frac{V_s\beta}{V_0}$	$0{\cdot}77\,\frac{V_s\beta}{V_0}$	*pH*
	Sun out, sky unclouded ...	11.30	3·5	59,500	—	—	—	—	—
A	Do. ...	11.53	—	62,000	180	0·29	1·0	0·8	6·0
„		11.55	3·7	62,000	—	—	—	—	—
B	Do. ...	12.8	—	62,900	340	0·54	2·0	1·5	6·0
„		12.12	3·8	63,200	—	—	—	—	—
C	Do. ...	12.29	—	63,200	220	0·34	1·2*	0·9	5·7
„		12.34	3·4	63,500	—	—	—	—	—
D	Sun unclouded, but dark cloud in east.	1.16	—	64,600	230	0·35	1·2*	0·9	6·1
„	Light clouds over sun ...	1.18	2·6	67,700	—	—	—	—	—
„	Do. ...	1.21	—	63,200	340	0·53	1·4†	1·1	6·1
E	Sun behind clouds and just obscured by wood.	1.34	1·0	33,000	<20	<0·06	<0·06	<0·05	5·6
F	Sun obscured, sky overcast	1.47	1·0	17,500	<20	<0·12	<0·12	<0·09	—

* Taking $\beta = 3{\cdot}4$. † Taking $\beta = 2{\cdot}6$.

Sept. 24th, 1925.—Area No. 3 (repeated). Base in open, near edge of cliff, 19 metres east from rail at edge of Cawsand wood, further north than No. 2. All values of β refer to base. δ_0 assumed to be 77 per cent., as before, since values of β, which would generally indicate a lower value, were rather erratic on this occasion. Wind N.W.

Area No. 3. Site.	Weather conditions.	G.M.T.	β	Illumination, metre candles. In open. V_0	In shade. V_s	Position of photometer in wood.	Percentages Daylight factor. Apparent. $\frac{V_s}{V_0}$	$\frac{V_s\beta}{V_0}$	Corrected. $0{\cdot}77\frac{V_s\beta}{V_0}$	pH
A	Sun clear, some cloud, mainly blue sky to east ...	10.45	4·7	46,500	—	——	—	—	—	—
		11.44	—	53,000	220	Horizontal ...	0·42	2·0	1·5	6·0
„	Do. ...	11.50	—	49,900	400	Sloping 45° N. ...	0·80	3·8	2·9	6·0
„	Do. ...	11.53	—	49,900	380	„ 60° N. ...	0·76	3·6	2·8	6·0
„	Do. ...	11.54	—	49,900	130	„ 45° S.E. ...	0·26	1·25	1·0	6·0
B	Do. ...	11.58	—	49,300	220	Horizontal ...	0·45	2·1	1·6	6·0
„	Do. ...	—	—	—	760	Sloping 30° N.W. ...	1·54	7·2	5·5	6·0
„	Do. ...	12.0	—	49,300	760	„ 60° N.W. ...	1·54	7·2	5·5	6·0
	Sun obscured by cloud ...	12.2	—	11,100	—	——	—	—	—	—
„	Sun clear, β relative to 12.2 ...	12.5	4·4	48,800	130	Sloping 30° S. ...	0·27	1·2	0·9	6·0
D	Sun clear	12.7	—	55,300	520	Horizontal ...	0·94	4·1	3·2	6·1
„	Do.	—	—	—	540	Sloping 45° N.W. ...	0·97	4·3	3·3	6·1
„	Do.	12.10	—	52,200	400	„ 30° S.E. ...	0·77	3·4	2·6	6·1
„	Do.	—	—	—	400	„ 45° S.E. ...	0·77	3·4	2·6	6·1
„	Do.	12.13	—	52,200	200	„ 75° S.E. ...	0·38	1·7	1·3	6·1
C	Sun clear, β relative to 12.21 ...	12.17	3·4	50,500	200	Horizontal ...	0·40	1·4	1·1	5·7
„	Very dark cloud	12.21	1·0	15,000	90	Horizontal ...	0·60	0·6	0·5	5·7
„	Sun clear, β relative to 12.21 ...	12.23	3·3	49,900	180	Sloping 30° N.W. ...	0·36	1·2	0·9	5·7
„	Do. ...	12.24	—	50,200	110	„ 60° N.W. ...	0·22	0·7	0·6	5·7
	Sun near wood, value of β	12.37	5·2	46,000	—	——	—	—	—	—
	fictitiously increased ...	12.41	5·8	49,300	—	——	—	—	—	—

TABLE 9.

Sept. 14th, 1925.—Area No. 4. Base near edge of cliff, on path, 13 metres east of rail at edge of Cawsand wood, further north than No 3. Values of β, save the last, refer to base No. 4. δ_0 = 65 per cent., assuming β = 4·1. Wind light, S.E.

Area No. 4. Site.	Weather conditions.	G.M.T.	β	Illumination, metre candles. In open. V_0	In shade. V_s	Position of photometer in wood.	Percentages Daylight factor. $\frac{V_s}{V_0}$	Apparent. $\frac{V_s\beta}{V_0}$	Corrected. $0{\cdot}65\ \frac{V_s\beta}{V_0}$	pH
A	Sun clear, very light clouds and thin wisps of clouds. Sun leaves Site A about 10.30.	10.26	4·1	45,400	—	——	—	—	—	—
		10.44	—	51,000	9,810	Horizontal ...	19·2	79	51	6·1
B	Do.	10.58	—	52,200	8,230	„ ...	15·8	65	42	—
C	Do.	11.13	—	54,700	880	„ ...	1·6	6·6	4·3	5·6
		11.22	—	51,600	630	„ 2 ft. above ground.	1·2	4·9	3·2	5·6
		11.26	—	51,300	2,300	Sloping 60° N. ...	4·5	18	11·5	5·6
		11.29	—	51,900	1,510	„ 60° E. ...	2·9	12	7·9	5·6
		11.32	—	51,900	630	Horizontal, 2 ft. above ground.	1·2	4·9	3·2	5·6
		11.34	—	52,700	630	Horizontal, on ground	1·2	4·9	3·2	5·6
D	Do.	11.49	—	55,000	7,560	„	13·7	56*	36	6·1
		11.53	4·6	54,700	—	——	—	—	—	—
	On wall of pier, sun out, clear sky.	1.40	3·3	52,400	—	——	—	—	—	—

* This and all other values calculated from β = 4·1.

Area No. 4, as in Table 9.

Site A.—Outside wood, about 6 m. from gate to wood, opposite cowshed, large patch of *U. dioica, Stellaria media* plentiful, grasses (*Poa pratensis*), large plants of *R. obtusifolius*; ground moist, *pH* 6·1; δ = 51. Sun leaves site about 10.30 in mid-September.

Site B.—Patch of *U. dioica* by hedge near gate, about 5 m. from A; soil apparently similar to A; δ = 42.

Site C.—Under, and to E. of, large *Castanea,* the first tree in the wood, above path near gate, aspect open to N. and E., *R. fruticosus,* well developed, *Arctium lappa, Lychnis dioica,* a little *H. helix,* mixed grasses ending, and *Bromus asper* just beginning; soil dry, *pH* 5·6; δ = 4·3, but up to 11.5 with photometer sloping.

Site D.—Inside shade of wood, but just clear of shade of overhanging branches of the *Castanea* of site C. Mainly mixed grasses, with clover, *Centaurea nigra,* and odd bushes of *R. fruticosus,* also *R. obtusifolius*; soil not so dry, *pH* 6·1; δ = 36.

The details concerning the illumination at the foregoing sites of area No. 4 are recorded in Table 9.

Discussion of Results.

From the foregoing, it may be seen that the interior of a mixed deciduous wood of this type receives only 1–2 per cent. of the diffuse daylight. Sites nearer the edge, where there is an appreciable outlook, though still within the wood, may receive larger amounts, up to about 20 per cent., if the maximum value is considered, as obtained by setting the photometer in the most favourable position. It is obvious that the diminution in light intensity is, in many cases, the factor which limits the penetration of plants into the shade, whereas others are unable successfully to compete for room if deprived of the advantages afforded by their ability to thrive in shade. Light, however, is not the only factor; change in soil reaction cannot be neglected; even the alteration from *pH* 6·0 to 5·6 is an increase of two and a half times in acidity; reduction in water supply may also be of importance, as the trees screen the ground from light rain, and their roots absorb much water; considerable weight must also be attached to protection from wind. Bearing the existence of the other factors in mind, it is nevertheless of interest to consider the light variation in relation, for example, to certain selected species from among those listed.

Thus, *Angelica sylvestris* and *Chrysosplenium oppositifolium,* both inhabitants of somewhat moist situations, were found in sites with daylight factors 42 and 34, and 16 and 1·9, respectively. *Rubus fruticosus,* of which the numerous sub-species thrive in the most diverse habitats, is recorded from nine sites, being more or less thriving with daylight factors 56, 42, 36, 34, 18, 16·5, and 4·3; but stunted and straggling on the ground with values of δ 2·9, 1·8, and 0·8. *Urtica dioica* was found dominant with δ 51, 42, and 37; a few plants occurred at two sites with δ 16, one plant only at each of two sites with δ 1·9. Olsen (1921) has stated that the nettle does best in half shade, with high nitrates, and relatively high water-content. *Lychnis diurna* was observed at δ 16, and at another site where δ = 4·3, rising to 11·5 with sloped photometer. *Rumex obtusifolius,* which thrives in open ground and gardens, was observed where δ = 51 and 36, and in stunted conditions where δ = 2·0 and 1·6. *Hedera helix,* on the other hand, found scantily with δ = 18, rambles as a dominant over all but the most densely shaded ground from δ = 4·3–0·8. The wood grass, *Bromus asper,* which seems never to occur in an open field, though it does on banks facing south, is a useful index plant; it has been noted with

δ 18 to 1·6 or δ (max.) 43–55, whereas the mixed grasses found outside the wood have not been found inside at lesser illumination than δ (max.) 11·5.

On comparing the illumination measurements with the general distribution of the plants, it seems that the daylight factor is a valuable index when expressed relative to the vertical illumination well inside the wood; where, however, there is an opening permitting the entry of light, the growth of the vegetation seems in better agreement with the reading of the maximum setting of the photometer expressed as a daylight factor.

It remains to consider the relation of the total intensity *I*, as previously defined, towards the character of the vegetation at the various sites. As may be appreciated from the earlier discussion, *I* cannot be ascertained with any accuracy just inside a wood, where the distribution of the lighting is very irregular, unless special measurements are made orienting the photometer in a number of directions; well inside the wood, however, in sites receiving illumination through the tops of tall trees and through distant small openings, it appears legitimate to consider the illumination as uniform—then $I_s = 2V_s$. If the conditions in the open are also such that $I_0 = 2V_0$, obviously the daylight factor, δ, is unaffected. If there is direct sunlight outside, its effect on the photometer at the base is allowed for in finding the "daylight factor" for the shaded site, this factor being defined with reference to diffuse light only.

It was hoped that it might have been possible to examine the various sites when the trees were leafless, but pressure of other work and illness have rendered this impracticable up to the present.

Summary.

Measurements have been made of the illumination in the shade and in the open, by a series of almost simultaneous determinations of the two values, by means of two photo-electric photometers and an apparatus previously described. The most satisfactory way of expressing the illumination at a shaded site, in relation to its flora, is as a percentage of the diffuse light in the open; this is termed the daylight factor. It is normally determined with the photometer horizontal, but at the edge of a wood the best correlation between plant growth and illumination is obtained by expressing the maximum reading, found by sloping the photometer, as a daylight factor. The total illumination, *I*, has been considered in relation to the vertical illumination, *V*, as measured. Data are presented concerning the distribution of various plants in terms of the daylight factor. This may be as low as 1–2 per cent. on the floor of a wood of mixed deciduous trees in autumn, and less than 0·06 per cent. in specially heavy shade under rhododendron. The photo-electric cells were sensitive to the blue portion of the spectrum. It is of interest to note that such values, 1–2 per cent., as are found in the wood are also found at depths of about 22–29 metres in clear water some miles off the coast outside Plymouth Sound.

In conclusion, the authors desire to express their indebtedness and to record their thanks to Prof. W. E. Thrift, for the loan of apparatus and for general laboratory facilities in the Physical Laboratory, Trinity College, Dublin; to Prof. J. Joly, for the loan of apparatus; to Dr. E. J. Allen, of the Marine Biological Laboratory, Plymouth, for the loan of apparatus, and for the facilities afforded at the Pier Cellars hut; and to the Government Grant Committee of the Royal Society for defraying the expenses of the portion of the apparatus necessary for extending its use to the study of vegetation on shore instead of in sea water.

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No. 26.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1925.

[Read April 27. Published separately September 21, 1926.]

The quantity of emanation issued during the year was 14,401 mc., as compared with 12,885 mc. in 1924, and 12,797 mc. in 1923. No change has been introduced in the laboratory technique. Detailed reports of the results of radium treatment sent in by two of the largest users of emanation are printed below.

A REVIEW OF THE RESULTS OF RADIUM TREATMENT OVER A PERIOD OF TEN YEARS (1916–1925).

By Maurice R. J. Hayes, M.D., F.R.C.S.I.

It does not seem possible to assess the value of radium in therapeutics by merely recording the number of cases treated and noting the results obtained within the brief space of twelve months, as this time is too short to observe the permanency of results, or to express opinions concerning cases that may have been treated shortly before the end of the year.

For these reasons, the cases treated during the past ten years, whose records are available, are now reviewed. The early and late results, in so far as it is possible to ascertain them, are recorded in every case, and they have been obtained either from the doctor in charge of the case or from personal observation.

Many of these are incomplete, however, because of the difficulty of "following up" cases. The majority of them have been referred to me by other doctors, and many of them are scattered in remote parts of the country; and very often one hears of them casually, months or years later, when they can neither be recalled nor identified in case records.

Table I.

Carcinoma of the Uterus.

No.	Age.	Cervix.	Body.	Duration.	Date of Treatment.	Result. Early.	Result. Late.
1.	60	—	X Fixed and enlarged	1 yr.	30/11/15 21/1/16	Normal size movable	Unknown
2.	50	X	X	1 yr.	14/12/15	Improved	Death 1 yr. metastases
3.	50	X	—	—	1/12/16	Improved	Unknown
4.	66	X	—	—	15/6/17	Unknown	
5.	63	X	X	2 yrs.	12/7/21 12/1/22 1/9/22 14/4/24 25/7/24	Improved „ „ „ „	Still living and doing well
6.	55	X	X	8 mos.	23/5/22	Hysterectomy 3 months later	Died Jan., 1923, metastases in liver

No.	Age.	Cervix.	Body.	Duration.	Date of Treatment.	Result. Early.	Result. Late.
7.	47	X	X	—	27/5/22 11/7/22 7/10/22	Improved „ „	Died May, 1923
8.	60	X	X	10 wks.	5/6/23 6/6/23		Hysterectomy on 7/12/23. Patient still well
9.	40	X	—	1 yr.	12/6/23	Improved	Unknown
10.	47	X	X	6 mos.	8/8/23 15/10/23	Much improved	Death metastases 1 yr.
11.	54	X	—	7 mos.	31/8/23	Improved	Died 6 mos. later metastases
12.	63	X	—	18 mos.	7/12/23	Improved	Unknown
13.	47	X	-	6 mos.	12/12/23	Improved	Died 6 mos. metastases
14.	41	X	—	6 mos.	8/1/24	No local recurrence	Died metastases Jan., 1926
15.	59	X	—	9 mos.	22/2/24		Death metastases 2 months
16.	45	X	—	9 mos.	25/3/24	Doing well in June, '24	Unknown
17.	38	X	—	1 yr.	13/5/24 25/6/24	Improved.	Died Dec., 1924, metastases
18.	37	X	—	4 mos.	25/6/24 29/7/24	Local improvement	August, 1924, general metastases, Died Sept., 1924
19.	65	X	—	6 mos.	13/11/25 21/1/25	Healed locally and given Deep Therapy	Doing well
20.	53	X	—	6 mos.	13/5/25 15/6/25	Healed locally and given Deep Therapy	Doing well
21.	—	X	—	6 mos.	4/8/25	Improved.	
22.	31	X	—	—	27/11/25	Improved.	Unknown
23.	—	Vaginal Vault	— — —	— — —	18/12/20 15/3/21 11/5/21	Much improved	
24.	45	Recurrence in Vaginal Vault 7 mos. after Hysterectomy	—	—	6/7/21	Unknown	Unknown
25.	57	Recurrence in Vagina 2 yrs. after Hysterectomy	—	—	11/7/21	Unknown	Unknown
26.	38	External Genitals. Recurrence 2 yrs. after excision	— — — —	— — —	13/1/21 19/2/21 21/3/21 5/5/21	Improved	Recurrence in 1925 after she had left Ireland
27.	45	External Genitals. Recurrence 9 months after operation	— —	— —	23/5/24 27/6/24	Not improved	Died August, 1924

Carcinoma of the Uterus.

In the table are given the patient's age; the site of the disease, whether the cervix or the body of the uterus; its duration; the date when treated, and the early and late results. All the cases were inoperable: their ages varied from 37 to 66 years. Most of them had symptoms of the disease extending over a period of six to nine months; but one sought relief within ten weeks, and in her case hysterectomy was done exactly six months after the first application of radium. Another patient had symptoms for two years before she sought advice, and she is still alive and doing well, after four and a half years.

In the twenty-seven cases recorded, the disease was primary in twenty-two, and recurrent in five. In three of the latter the disease reappeared in the vagina following hysterectomy, and in two there was local recurrence after excision of the primary focus in the external genitals.

In the twenty-two primary cases, the cervix only was affected in fourteen, the cervix and body in seven, and the body alone in one.

The *immediate results* of treatment were local improvement in all cases, as evidenced by cessation of discharge and hæmorrhage, and the cicatrisation of malignant ulceration, and the formation of healthy scar tissue.

As regards the *final results*: four died from metastasis within six months or less, three within one year, one after five years. In one case hysterectomy was done three months after treatment, but she died of metastasis five months later. In another, hysterectomy was possible six months after the first treatment. This patient is very well, and there has been no recurrence of the disease for two and a half years.

One is alive for four and a half years, but she is not free from the disease.

There is no late record of six cases.

In four cases of cervical carcinoma treated within the past year, the disease has healed locally. In two of these, radium treatment has been supplemented by High Voltage X-rays (200,000 v.), and it is intended to treat the other two in the same manner.

The favourable results of radium treatment in the foregoing cases are as follow:—

1. Local arrest of the disease, with the formation of a healthy scar.
2. Arrest of toxæmia, hæmorrhage, fœtid discharge, and pain.
3. A temporary improvement in general health, and prolongation of life in comfort (4½ years in one case).
4. Hysterectomy became possible in two inoperable cases.

In the treatment of advanced and inoperable carcinoma of the uterus, radium is of undoubted value. It will give relief where nothing else avails. It will arrest the disease locally; and, if metastasis has not already taken place, it may *cure* it.

It will be observed that the known cause of death in every case where it is established in this series is metastatic disease; and it is reasonable to infer, because of the duration of the symptoms, that secondaries were already present before treatment was begun.

Nothing was observed following the treatment in any of the cases to confirm or refute the contention that radium stimulated the disease, except perhaps in No. 15, who was reported as having died of metastatic disease within two months, although there was no evidence of secondaries in this case when she was treated.

Since the gamma rays of radium, like other light rays, conform to "the

law of inverse squares," and their effects at 3 cm. distance are only one-hundredth part of what they are at the surface of the applicator, they cannot destroy outlying metastases.

The technical and mechanical difficulties of thoroughly irradiating the pelvic cavity are so great, whether by methods of implantation or surface applications, that one cannot confidently hope to destroy metastases by radium, or to accomplish *the ideal* in the treatment of uterine carcinoma, viz.: "A Röntgen Wertheim."

The chief advantages of radium treatment are its rapid and thorough local effects, the ease with which it can be applied, and the slight systemic disturbance which follows correct dosage. It is, however, a dangerous agent in the hands of the inexperienced. The damage that may accrue through carelessness in handling and storage of it is very real, and the injury that may befall patients through ignorance of the best known methods of applying it may be very serious. These essentials are sometimes forgotten, or they are realised only when it is too late.

When the disease has extensively involved the rectum or bladder, fistulæ of these viscera may occur during the natural process of healing. This is a complication to be anticipated.

Mode of application and dosage.—Radon is suitably packed in brass tubes 1 mm. thick. These are placed in the cervical canal, or in the cavity of the uterus, according to the exigencies of the case. In cervical carcinoma, needles are sometimes embedded circumferentially around the os while the tube is *in situ*. When the cervix is eroded, and cannot be identified, or is impermeable, needles alone have been implanted; or, if this is not possible, the brass tube alone is placed in contact with the eroded surface. In all cases the vagina is thoroughly packed with iodoform gauze in order to hold the radium in position, and to keep the bladder and rectum as remote as possible from it.

The dose given at one time has varied from 600 to 2000 millicurie-hours, according to extent and conformation of the lesion, and the general condition of the patient. The smaller dose is, if possible, repeated within three to four weeks.

Whether given by a single or frequent repeated applications, the aim has been to administer a total dose of from 1800 to 2000 millicurie-hours.

From the relatively small number of cases, and the varied conditions under which it is found necessary to treat them—histological examinations of specimens are not always available—it is not possible to draw any conclusions as to the relative merits of small repeated, *versus* solitary large doses.

The systemic reaction, as manifested by a rise in temperature, malaise, nausea, and sometimes vomiting, is greater following a single large than repeated small doses. The severity of the reaction seems to be directly proportional to the amount of sepsis which is present, but it usually disappears within twenty-four hours.

Menorrhagia and Metrorrhagia.

Unmarried, aged 28.—For six years there was copious menstruation at regular intervals of sixteen days. It lasted from four to six days. There was no discernible abnormality of the uterus or ovaries, and there was no response to any form of treatment.

With the hope of controlling the symptoms, and, if possible, without establishing an artificial menopause, an intra uterine application of 300 millicurie-hours was given on February 15th, 1921. Following this, the duration and

the frequency of the periods were decreased, and the treatment was repeated on the 17th June, 1921. When seen again the following September, the patient said that she had not menstruated for two months until a few days previously, and then only for 1½ days.

The effect in this case has been longer intervals between, and shorter duration of the periods, but a very severe headache accompanies them.

Unmarried, aged 23.—The patient was well-nourished, but anæmic. Since the age of sixteen she had been unwell every ten to fourteen days for about ten days, and sometimes a fortnight at a time. She had been curetted three times, and her appendix and part of both ovaries had been removed, but without any beneficial effect. 18th September, 1920, she was given 140 millicurie-hours intra uterine through 1 mm. of brass. The patient derived so much benefit that she declined a repetition of the treatment. She is still quite well.

Married, aged 40.—Irregular frequent menstruation, and cystic left ovary. 15th March, 1921, she was given an intra uterine application of 300 millicurie-hours.

Result—improved—no recent report.

Rodent Ulcers.

Eighty-five cases have been treated from 1915 to December, 1925. Of twenty-three cases treated up to the end of 1923, all were apparently cured, except one, which invaded the orbital cavity. This case is still under treatment.

In sixty-two cases treated during the past three years, the ulcers have healed in all, except one, in which the cartilage of the ear was invaded. The external ear was excised in this case. The technique employed is the same as that described in the last report.

In this group of eighty-five cases, it is not suggested that all are still alive. Many of them were old and infirm, and they have died from the diseases incidental to old age. There has been no recurrence of the ulceration, however, in any of them.

[Table II.

TABLE II.

Oral Carcinoma.

Age.	Site of Origin.	Glands.	Recurrence. Local.	Recurrence. Glands.	Result.
76	Tongue	—	X	X	Death 4 months.
60	Tongue	X	—	—	Death 4 months.
56	Tongue	—	—	X	Death 3 months.
51	Floor of mouth	X	—	—	Cured 10 years.
54	Tongue	X	—	—	Death 4 months.
53	Floor of mouth	X	—	—	Unknown.
37	Lip	—	—	X	Cured 5 years.
39	Tongue	X	—	—	Death 3 months.
41	Floor of mouth	X	—	—	Death 4 months.
38	Tongue	—	—	X	Cured 5 years.
45	Nasal cavity	—	—	—	Death 4 months.
72	Lip	—	—	—	Unknown.
63	Tonsil	X	—	—	Death 3 years, metastases in liver.
54	Floor of mouth	X	—	—	Death 2 years, metastases.
68	Lip	—	—	X	Unknown.
61	Lip	—	—	X	Death 7 months.
65	Lip	X	—	—	Death 2 years.
55	Tonsil	X	—	—	Death 4 months.
49	Palate	X	X	X	Recurrence after 3 years, again under treatment.
50	Tongue	X	X	X	Death 2 months
74	Cheek	X	X	X	Death 9 months.
75	Lip	—	X	—	Death 9 months, Pneumonia.
68	Lip	—	—	—	Unknown.
37	Tonsil	—	—	—	Healed 9 months.
58	Lip	—	X	—	Healed 2 years.
45	Lip	—	—	—	Healed 21 months.
60	Cheek	—	—	—	Healed 19 months.
37	Floor of mouth	X	—	—	Death (?) 4 months.
63	Lip	—	—	—	Healed 15 months.
58	Tonsil	X	—	—	Healed 14 months.
61	Tongue	X	—	—	Unknown.
55	Palate	—	—	—	Healed 13 months.
60	Tongue	X	—	—	Doing badly after 6 months.
75	Lip	—	X	X	Improved 6 months.
71	Floor of mouth	—	X	X	Not improved.
74	Lip	—	—	—	Under treatment.
29	Epulis Jaw	—	—	—	Under treatment.

The thirty-seven cases in this group are comprised as follows:—

Lip	12
Tongue	9
Floor of Mouth	6
Tonsil	4
Cheek (Mucous Membrane)	2
Palate	2
Nasal Cavity	1
Jaw (Epulis)	1

Lip.—Five of the lip cases were primary, without palpable glands. One of these healed in 21 and one in 15 months. One is under treatment, and two cannot be traced.

One primary case, with enlarged glands, died two years after treatment.

In two, there was a local recurrence following operation. One of these died from pneumonia six months after treatment—the ulcer had disappeared—and the other has been well for two years.

In one case, there was a local post-operative occurrence, with enlarged glands. This case is improved six months after treatment.

In three cases there was a recurrence in the glands. One of these has been well for five years. One died after 7 months, and one cannot be traced.

Tongue.—Five were primary, with enlarged glands. Three died within four months. One is doing badly, after six months. One, result unknown. In two there was recurrence locally and in the glands, following operation. One died after two and one after four months.

In two there was a post-operative recurrence in the glands only. One has been well for five years; the other died within three months.

Floor of the Mouth.—One has been quite well for ten years; one died from metastases after two years. Two died within four months; one is not improving, and one cannot be traced.

Tonsil.—One died after four months; one after three years; one has been well for nine months, and one for fourteen months.

Mode of Treatment.—Small superficial ulcers on the lip are treated by surface applications with filters of 2 mm. aluminium. Needles are embedded if there is much induration, or if the ulcer is of the fungating type. Dose, 60 millicuries per square centimetre. When the growth is located in the tongue, the tonsil, or the floor of the mouth needles are embedded in and, if possible, around it.

The submaxillary and cervical lymphatics were formerly treated by surface applications filtered through 2 mm. of brass—1800–2000 millicurie-hours, 3 cm. distance.

When there were large secondary tumours in the neck, in addition to surface applications, needles about 2 inches long were pushed through the skin into the substance of the tumour; but this method has been abandoned, because the needle punctures did not heal; serum or necrotic tumour-tissue oozed through the openings, which became infected, and all cases treated in this way did very badly.

The passing of needles upwards through the skin in order to treat malignant ulcers of the floor of the mouth has been also abandoned for the same reason. Cellulitis is very liable to follow this procedure, and salivary fistulæ have frequently formed.

The implantation of needles in the growth inside the mouth has not been followed by the same bad results, even though the tissue immediately surrounding the needle may have received an overdose, and it is recognisable as a small, white slough, which persists for many weeks after treatment; but these are not troublesome, and they do not persist.

In the writer's experience, high-voltage X-rays have been more effective than radium in checking glandular metastases. He attributes this to their more diffuse action, and the more homogeneous deep-seated radiation which can be obtained by them. It is also conceivable that the biological effects of X-rays and radium may vary with the histological structure of the tissues. In the light of our existing knowledge, and on the general principle that combined remedies having similar effects are more certain in their action than a single dose of any one of them, there are very good reasons for their combined use until, with more knowledge and experience, our methods become less empirical and more rational.

After Treatment.—It is always important to attend carefully to the toilet of the mouth before and after treatment. There must be a liberal use of peroxide of hydrogen, listerine, or other antiseptic mouth washes.

Miscellaneous.

Radon has been employed in the treatment of three cases of *Branchial Cleft Carcinoma* in elderly men. All were advanced, and the tumours were large. One of these, who was treated in 1915, was not improved. Another, treated in 1915, got temporary relief; he died seven months after the treatment. The third was treated by low-voltage X-rays and radium in 1919 (surface applications: 3000 millicurie-hours filtered through 1 mm. lead: 6 fields: cross fire). There was considerable reduction in the size of the tumour, and pain disappeared. He died a year later.

Carcinoma of Rectum.—M., aged 43, who was treated in December, 1923, is still apparently free from the disease, and able to attend to his regular work. This case is recorded in my last report.

Keloid.—Four cases are apparently cured—one five years, one four years, one three years, and one two years. The cosmetic results are excellent. All were given surface applications through 2 mm. aluminium—40 millicurie-hours.

Warts.—Very many of these have been removed successfully. For the soft, redundant, cauliflower type, a surface application of 30 to 40 millicurie-hours filtered through 2 mm. aluminium has been found sufficient; but for the hard, indurated, horny variety a somewhat larger dose is required.

Villous Papilloma of Bladder.—M., aged, 63. Papillomatous growths, chiefly on fundus of bladder; latter contracted; persistent hæmorrhage; voiding clots and villi. Prostatic hypertrophy. 19th February, 1921: 18 millicuries of radon fixed by sterile wax in the end of a metal catheter which was passed into the bladder. Its position was altered every two hours. It was left in the bladder for eight hours. 9th April, 1921.—The treatment repeated, using specially devised catheter, having a filter of 1 mm. of brass. The dose administered was 140 millicurie-hours. This catheter was so constructed as to allow the bladder to empty without removing it. Following the treatment, the symptoms disappeared. There was no further hæmorrhage, and there was considerable reduction in the size of the prostate. When last heard of, in 1924, there was no recurrence of the symptoms.

Cases Treated with Radon during 1925.

The cases which have been treated during 1925 are as follows:—

Carcinoma of Tongue	2
Warts	2
Papilloma Palate	1
Rodent Ulcers	22
Carcinoma of Rectum	1
„ „ Cervix Uterine	3
„ „ Lip	2
„ „ Breast (Primary)	1
„ „ „ (Recurrent)	1
„ „ Penis	1
Epithelioma Face	3
Carcinoma of Floor of Mouth	1
Keloids	1
Metastatic Hypernephroma	1
„ Endothelioma	1
Pigmented Mole (Hairy)	1
Epulis Jaw	1

These cases are included in the body of my report.

REPORT OF ONE YEAR'S RADIUM WORK CARRIED OUT IN 1925.

By Walter C. Stevenson, M.D., B.Ch., D.P.H.

Introduction.

The number of patients given radium treatment during 1925 was 188; of whom 100 suffered from malignant diseases, and 88 from non-malignant conditions. This is the largest number of patients treated by me during any one year. My source of energy was principally radium emanation, supplied by the Royal Dublin Society in glass capillaries. In the majority of cases this has been supplemented by the use of 53·93 mg. of radium element in six capillary cells; 5 of 0·2 mm. platinum, containing about 10 mg. of element each; and one of 0·2 mm. steel containing about 5 mg. of radium element (10). As the radium cells and the radium emanation capillaries are about the same size, they are used indifferently, and often in the same containing tube.

In carrying out the work, the general attitude adopted has been to treat all patients referred to me suffering from cancer, irrespective of the hopelessness of the case. These patients were mainly treated in Dr. Steevens' Hospital, and a few in other general hospitals, at the request of their staffs. Unfortunately, a number of these patients, mainly those from the country, pass through my hands, and are lost sight of, and, though the immediate results appear satisfactory, my records go no further.

The treatment of non-malignant conditions was originally undertaken by me on experimental lines about ten years ago for disabling, but normal, scar tissue (not keloids) resulting from gunshot wounds, which interfered with function, and had ceased to improve by the routine treatment in the massage department

of military hospitals (6). It was found by careful measurements and observations that painful scars became less tender and less adherent, the movements of joints were improved, and there was frequently marked improvement in the nutrition of the limb where the wound involved the neighbourhood of large vessels and nerves (7 and 8). By administering larger doses now, these beneficial changes are even more apparent than they were some years ago. Gradually radium treatment has extended to other non-malignant conditions, which vary greatly in their nature. That it is a powerful agent in combating chronic infections has been proved. It is particularly useful in relieving symptoms and hastening recovery in tuberculous peritonitis (9), and would appear to be of use in pulmonary pthisis—at any rate, in some cases. As a rule, radium is only used for this work when it is not required for malignant cases; and as my supply is in constant use, day and night, and is simply moved from one patient to the next, opportunities frequently occur for the treatment of non-malignant conditions. Otherwise, the energy would be lost, and opportunities for observing the effect of this form of treatment would be wasted.

I early realised that dosage (4 and 5) and technique (1, 2, and 3) play an all-important part in successful treatment, and it is only by modifying technique from time to time that improved results can be expected. It is, therefore, important to know the doses administered, the treatment and technique employed, and the physical condition for which it is used. In considering dosage, one requires to know accurately the amount of energy that has been expended on the patient. To form a just estimate of this it is necessary to record: (*a*) the amount of radiant energy available during the treatment; (*b*) its distribution; (*c*) the amount of screening; (*d*) the distance of the radium from the tissues treated.

It is essential for comparing doses to have some standard method of measuring energy used up during treatment. My own plan for many years has been to record it by the amount of emanation lost during the treatment. When I used emanation alone I found this directly from a table which has been previously published (5). It is very convenient to be able to ascertain at a glance the activity of a supply of emanation at any hour, and to count on the table the number of hours it will take to lose, say, 9 mcs., *i.e.* to give a dose of 9 med., instead of having to calculate the length of exposure which will be required to give the same dose—*i.e.*, 1200 millicurie-hours. In the case of radium element I use the same method, and reckon the amount of loss as constant at $\frac{3}{4}$ per cent. per hour in mcs. of the amount of radium element present. With radium emanation, the percentage loss per hour is the same, *i.e.*, $\frac{3}{4}$ per cent.; but the actual loss diminishes slightly from hour to hour as the activity of the emanation decreases. The loss in millicuries or the disruption or destruction of emanation during treatment (med.) can easily be converted into milligramme-(mgh.) or millicurie-hours (mch.) by dividing by 3 and multiplying by 400, as 3 med. = 400 mch. or mgh. In whatever way the amount of energy used up during treatment is calculated, it gives no indication of the amount of energy available for absorption during the treatment unless the particulars of distribution, screening, and distance are detailed as well. For instance, my treatment is mostly carried on with gamma rays, but I find in many instances it is advisable and economical to use beta rays as well, in which case the amount of energy available for absorption is very much increased.

The essence of treatment of malignant disease is to give as big a dose as possible to destroy malignant cells, without permanently injuring healthy tissues, which we have to depend upon for the ultimate cure.

The crux in radium treatment is the short distance from the radiating

centre to which this dose reaches—in other words, the difficulty of uniform radiation. The idea of my whole technique is to obviate this difficulty to the greatest possible extent. There are three physical means of minimising the effect of distance, each of which I have utilised, as described below. They are:—

I. By using the maximum number of foci of radiation; each focus being mainly responsible for the efficient treatment of the malignant cells in its vicinity.

II. By screening off the non-penetrating rays where they are undesirable, so that there will be the minimum overdose in the immediate vicinity of the focus, with efficient treatment in the areas between the foci.

III. By employing distance pieces, so that when the rays reach the nearest cells they are already, to some extent, more uniformly distributed.

I. Radium is used either on the surface or surrounded by the natural cavities of the body, or actually embedded in the malignant tissue in needles or tubes. The best and most efficient treatment is obtained by combining surface and deep treatment, if possible carrying out both at the same time. The surface area of radiation is increased by spreading out the maximum number of capillaries available in boxes or surface tubes, so as to cover as big an area at the same time as is consistent with uniform and efficient radiation. In extensive disease, the skin surface is marked out in areas corresponding to the size of the applicators. When one area is efficiently treated, the adjacent areas are dealt with. The applicators are moved from one area to another, taking care that there should be no overlapping, and each area is given as big a dose as the skin will stand. In dealing with natural cavities, such as the uterus, the radium is first applied to the fundus of the uterus; and when this has been efficiently treated, the tube, which is screwed into a wire marked in centimetres, is pulled out the required distance, usually two or three cms. (1·5 to 2·0 cms. being the length of the capillaries and radium cells), and the next areas similarly dealt with. This procedure is repeated, until the whole area requiring radiation is treated. The wire attached to the tube projects from the skin surface and passes through a clip arrangement, which is fixed to the skin, and is immobilised, so that, by the mark at which the wire is clipped, and from measurements taken before treatment is commenced, the exact position of the radium is known at any time. When the needles or tubes are inserted into a malignant growth, six to twelve of them are put in parallel to one another, about equal distances apart, and made to traverse the whole malignant area requiring treatment, and dealt with in a similar manner to the tube in the uterus—that is, when one part is efficiently radiated, the tubes or needles are pulled out and left till the next part is efficiently treated, this procedure being repeated until the whole tumour is radiated. As these tubes and needles are sometimes inserted twelve or more centimetres through a growth, the wires or extension pieces used by me are made in short sections, to screw into one another. As the needles or tubes are withdrawn, the extension pieces which project beyond the skin become redundant, and are removed so as to cause as little inconvenience to the patient as is possible, and to minimise the risk of his movements disturbing the position of the radium.

II. With regard to screening, I sometimes use bare emanation capillaries, which is practically only sufficient to cut off the alpha rays. At other times I use serum needles, which have a screen of only 0·1 mms. of steel; and when the radium cells are used in their special needles, the screen is 0·2 mms. of

platinum and 0·4 mms. of steel needle. The tubes used for inserting in the tumour are 5 mms. and 6 mms. external diameter, and have 1 mm. brass walls. The brass tubes employed in the uterus are 7, 8, 10, 15, and 18 mms., and in the rectum and vagina, 26 mms. external diameter, and 2 mms. thickness of wall. Sometimes the screening of these tubes is increased by a cylinder of lead of 1 or 2 mms. thickness inside the tube of brass. For a number of years I have employed for surface treatment brass boxes, as previously described (6, 7, and 10), having an internal area of 3 by 2 cms., and a thickness of 3 mms., as a screen. I found that these boxes were heavy, and sometimes inconvenient to adapt to the contour of the part to be treated. On account of this, I have recently adopted brass surface tubes, 90 of which I had made, and find them much more convenient than boxes for surface application. Each is capable of containing one to three emanation capillaries, or a radium cell. They are of 5 mms. external diameter, and have walls of 1·75 mms. of brass, and are 3 cms. long. An eyelet wire is screwed into either end, so that they can be strung together on a thin wire or thread, either with "separation pieces," of measured length between them, so as to cover a larger area, or in apposition with one another—*i.e.*, that is, the capillaries are 5 mms. apart. The separation pieces are made of paraflex, a rubber or celluloid tubing used for insulating in wireless work. These surface tubes are sometimes used with one or more connecting pieces, so that they can be screwed together in a line, and by arranging these sets of tubes parallel to one another, they have been found useful in vaginal work. When fixed to the convex side of a strip of lead bent into a longitudinal trough, the tubes can be retained in apposition with the diseased part of the vagina by packing gauze against the concave surface of the trough, an arrangement which also protects the parts which do not require intensive radiation.

III. The employment of distance pieces, their variety, and the rationale of their use, in increasing the efficiency of treatment, is usually not sufficiently recognised as of importance in technique. In literature, very little attention is paid to the external diameter of the tubes, but rubbers and other substances are described as covers to cut off secondary radiation from the metal of the radium-containing tube. Provided the primary beta rays are screened off by the containing tube, the use of further packing is only efficient, but of great importance as a distance piece. These coverings give off more secondary rays than any metal, except lead (see, for instance, Poole, Sc. Proc. R.D.S., 1922, 16, p. 467), but this disadvantage is more than counterbalanced by the effect of distance. When using standard boxes for surface application, I found erythema of the skin was produced with a dose of 1·8 med. when the box was against the skin—*i.e.*, the distance was 3 mms. brass and the thickness of the glass capillary. When using 2 or 3 mms. paper or leather as a filter, the dose was 3 med. at a distance of 6 mms. Later, when I used as distance pieces empty match-boxes, which had very little screening effect, but kept the radium 15 to 18 mms. away from the skin, the dose that produced the same effect on the skin I found to be 9 med.

When introducing radium into a natural cavity, the larger the tube I can use the less concentrated will be the dose in the immediate vicinity of the tube. The exposure can, therefore, be longer, and a greater effect is produced some distance from the tube. In practice, some structures may be regarded as distance pieces. For instance, a cancerous uterus is a distance piece to protect the intestines, &c.; and as the products of destruction can escape to the surface, it minimises the risk from absorption. When, however, the tube is withdrawn into the vagina, a dose which is safe in any part of the uterus would damage

a healthy vagina to such an extent that recto-vaginal and vesico-vaginal fistulæ would probably result. Therefore, the radium-containing tube, as soon as it, or part of it, leaves the external os, is made to enter the centre of an aluminium tube, which acts as a distance piece. The aluminium tubes I use are 4·2 cms. long, and of 35 mms. or 28·5 mms. external diameter, according to the size of the vagina. When the radium tube, with its extension wire projecting at the surface, is in position in the uterus, the aluminium distance tube is threaded over the wire, and pushed up against the cervix, so that, when the radium is withdrawn from the uterus, it cannot be nearer the vagina wall than 15 or 11 mms., according to the size of the aluminium tube. Up to two years ago I used in a similar manner as a distance piece a section of a large-size candle, perforated through the centre. Before that, up to 1920, when the supply of emanation was small, I used steel needles containing emanation capillaries inserted into the cervical tissues alone; and I still employ them or radium needles, in combination with pure gamma-ray treatment, in suitable cervical cases.

When I am treating a fungating mass on the surface, I insert radium needles or tubes, with the minimum screening, into this degenerating and useless tissue with the idea of absolutely destroying it; or, at any rate, disregarding overdose, provided the malignant tissue at the base, where healing would naturally take place, should get an efficient dose. Usually, these fungating masses are very hæmorrhagic. Immediately after such a treatment, the redundant tissue can be curetted away without danger of hæmorrhage, and the raw surface, if it has received a sufficient dose, heals up with as good a scar as is possible to obtain. With this procedure, the redundant tissue simply acts as a distance piece, and when dealt with in this way, the risk of dissemination and of hæmorrhage by surgical interference is reduced to a minimum.

A.—Cancer of Breast.

Summary of Cases.

Nine cases of cancer of the breast were treated, four for recurrence after operation (Cases 3, 4, 6, 9), in one operation and treatment were combined (Case 2), the remaining four were inoperable (Cases 1, 5, 7, 8). Five of these cases are apparently cured (Cases 2, 3, 6, 8, 9). One was improved (Case 5), in one the disease has progressed slightly (Case 1), as far as I know. One has been lost sight of (Case 4), and one died of bronchitis soon after treatment (Case 7).

The technique which I believe gives the best results in inoperable cancer of the breast is the insertion of 6 to 12 needles or tubes, or both, into areas where the disease is evident, combined with surface radiation. In cases of recurrence, where extensive operations have been done, there is usually not sufficient tissue left for the insertion of radium, in which case surface radiation is alone possible. The positions of needles and tubes are changed as before described. When tubes are used the skin is punctured or stabbed with the point of a knife, and the tube, with the trochar point screwed into one end, and the extension wire into the other end, is simply pushed through the tissues to the far end of the tumour and withdrawn the length of the radium as each area is sufficiently radiated, till the whole tumour is systematically treated. As the radium is never allowed to remain less than half a centimetre from the skin surface, the puncture wound in the skin heals without further trouble. The surface application is applied either in brass boxes 3 mms. thick, internal

area 3 × 2 cms., with emanation capillaries placed parallel to one another inside, or with emanation capillaries in the surface tubes. With the tubes, instead of match-boxes, felt 15 to 20 mms. thick acts as a distance piece and extra filter for the radium, as with the tubes the rays are filtered by 1·75 mms. brass, while with the boxes the filter is 3 mms. brass. The amount of radium used up is 1 to 1·5 mcs. per square centimetre, or 6 to 9 mcs. per box area.

As an example of combined radium and operative treatment in a so-called inoperable patient (Case 2), the following record will illustrate the rationale and technique employed:—A patient was admitted to Steevens' Hospital with a tumour about the size of a fist in the breast, in the line of the right anterior axillary fold. This was fungating, foul-smelling, excavated in the centre, and firmly adherent to the chest wall. There were glands below and in the axilla, but none could be felt above the clavicle. The fungating mass was very hæmorrhagic. From previous experience I have realized the danger of administering a severe radium dose and then performing an extensive operation while the patient still had constitutional reaction. If the operation is done first, when it must of necessity be an incomplete one (for instance, in this patient the chest wall would have had to be removed to get beyond the disease), the risk of dissemination by operation would be serious. I determined here to radiate excessively the portion of the obviously malignant growth which I intended to remove, and which proved to be an encephaloid carcinoma, so that the malignant tissue in this area which I could not remove would have had a lethal dose before it was interfered with surgically. On the 24th October last, 16 surface tubes containing 91·4 mcs., divided evenly among the tubes, were put into the cavity and pushed into the surrounding fungating mass without any anæsthetic. A couple of mms. thickness of gauze were first placed at the bottom of the cavity against the chest wall to prevent over-exposure in this area, which required efficient treatment, but not an overdose. The overdose of the remaining part was immaterial, as I intended to remove it. At the same time two boxes were placed without any distance piece on the glands under the axilla, as I intended to remove the glands with the skin over them. On the 26th October, 46 hours later, the fungating mass still containing the radium, the glands mentioned above, and some further up in the axilla, and half the breast, including and beyond all the indurated portion, were removed without encroaching on any septic area. The whole of the breast was not removed, as I expected that the skin brought together over the originally diseased area might not unite owing to the septic condition of the part, and the intensity of the radiation it had received, and I intended to use the skin over the remains of the breast to cover this area subsequently. The 16 surface tubes, after being cleansed and sterilised, were pushed up into the axilla and separated from the axillary vessels by 1 cm. of gauze, and from the anterior axillary fold by 3 or 4 thicknesses of gauze, and left there for 24 hours. The skin incision was closed over the breast area, but a small gap remained just at the axilla, and two or three stitches were not tied till the tubes had been removed from the axilla. I considered it important to treat the axilla, which had not been previously radiated, immediately it had been incised, so as to minimise the risk of dissemination from this area. Although the patient stood the operation extremely well, and did not appear to suffer from much shock, I considered it wiser not to continue the radium treatment at once. On the 6th November the chest below the clavicle, comprising an area of 14 by 2 cms., was treated by surface application for 42 hours, and the applicator was applied above the clavicle for 60 hours. On the 24th November the area at the top of

the incision where the skin had not been quite brought together was treated for 34 hours, and a similar dose was given along and in front of the healed skin incision, including the part of the breast that was not removed for 37 hours. Three days later the side of the chest was treated. The total amount of energy used up in this case was as follows:—

Before Operation.

16 surface tubes inserted into fungating mass, area 6 by 5 cm.	26·7 med.
Surface treatment glands, area 7 by 2 cm., distance 3 mm. brass screen	5·3 med.

At Operation.

16 surface tubes, area 8 by 2 cm., inserted into operation wound axilla	10·5 med.

After Operation.

Surface treatment at 20 mm. distance—

Below clavicle, area 14 by 2 cm.	28·1 med.
Above clavicle, area 14 by 2 cm.	26·9 med.
Over upper part of incision, area 9 by 9 cm.	22·6 med.
Over lower part of incision and breast in front of it, area 9 by 9 cm.	22·5 med.
Anterior axillary fold, area 10 by 2 cm.	18·7 med.
Surface beta-ray treatment, area 2 by 6 cm., 0·25 mm. lead filter, distance 3 mm. over unhealed area to stimulate formation of epithelium	0·5 med.
Total,	183·1 med.

Equivalent to 24,400 mc.-hours.

This patient first noticed the growth in December, 1924. It broke down in June, 1925. She was 61 days in Steevens' Hospital. When she left there was no sign of induration or thickening, most of the operation scar had healed by first intention. A small area under the axilla, which had not been originally covered with skin, still required dressing when she left hospital. She wrote twice since, last time in the end of February, stating she was quite well, and able to do her house work. One of the most satisfactory results of the treatment is that, in spite of the septic condition of the breast and the malignant nature of the tumour, the whole skin incision, which could be brought together at the operation, healed up at once without the least signs of recurrence or sepsis. I advised this patient to return for further treatment, but she has not done so.

Of the remaining four patients who have done well, in one (Case 3) the left breast was amputated in November, 1924; there was a lump about the size of a tangerine orange situated in the scar, some thickening on the axilla, a feeling of resistance in the neck, and she suffered a good deal of pain. This was treated by surface radiation alone, from the 22nd to 26th June, 1925, receiving altogether a dose of 167·2 med. by surface boxes on the side of the neck, in the axilla, and over the tumour, about 1 med. per sq. cm. at a distance of 15 mms., match-boxes being used as distance pieces. She writes in March, 1926, to say that she is perfectly well, and that the local surgeon can find no

trace of the disease. The third patient was operated on in October, 1924 (Case 6). She had two malignant ulcers on the site of the scar, together about the size of the palm of the hand, some glands in the axilla, and oedema of the arm which had been present since the operation. She was treated from the 9th to the 13th October, 1925, over the ulcer, in the axilla, and above and below the clavicle. After the treatment there was a good deal of redness and superficial ulceration of the skin which lasted about 3 months, but when last seen in the beginning of March all induration had disappeared, the ulcers had healed, and the pain which she had been suffering before treatment was gone. The dose she received was 155·4 med. at a distance of 15 mms. and about 1·0 med. per square cm. Another (Case 9) was similar, and had been operated on five months before her treatment in October, had some glands in the neck and small ulcers on the site of the scar. She received similar treatment over the shoulder, neck, axilla, and on the ulcers. Dose 162·8 med., 20 mms. distance, and about 1 med. per sq. cm. Two months later she reports that she is quite well, having first written to say her skin was very sore. The last patient (Case 8) was first treated in May, 1923, for a malignant mass involving the left nipple, which was ulcerated. She was treated altogether five times by surface application, and although the ulcer had nearly healed up, there was still a large amount of induration remaining when last treated in January, 1925. As a result of treatment the whole indurated area sloughed, destroying the skin over an area about the size of the palm of the hand, leaving a fairly deep raw surface which gradually filled in and healed with a soft scar. The area which ulcerated received a dose of 1 med. per sq. cm. at 15 mms., match-boxes being used as distance pieces. One of the breast cancers which has not done so well (Case 1) received surface treatment alone, as she would not submit to the insertion of needles. The ulcer and induration has been kept in check since June, 1923, but had extended when last seen in the end of 1925. She would never enter hospital to be treated and dealt with efficiently. Another patient (Case 4) with recurrent glands and tumour six years after operation, who received surface application, by the methods described, has not been heard of since she left hospital. A patient of 77 years with extensive disease of the breast, axillary glands, and glands of the neck, returned home and died a fortnight later from bronchitis, to which she was subject. During the week she was under observation and treatment the oedema of the upper limb from which she suffered had decreased one inch at the arm, an inch and a half at the fore-arm, and one inch round the hand. A patient who improved considerably with radium treatment (Case 5) had a lump about the size of a tangerine orange in the outer quadrant of the right breast, with small glands in the axilla. After receiving a dose of 167·5 med., the lump became free from the skin, and the glands in the axilla disappeared. When treated again in August, that is ten weeks later, the lump was slightly smaller, was not adherent to the skin, and the movements of the arm were freer and did not cause pain or discomfort. On the second occasion she received a dose of 170·4 med., and it is probable that the course of the disease has been checked. I regret that on account of the heart condition of this patient it is considered inadvisable to insert needles and tubes into the growth, in which case I believe a much more marked effect would have been produced.

The cases of cancer of the breast have been gone into fairly fully, as I have been able to trace them, also as it serves to illustrate the nature of surface treatment I have employed in dealing with malignant growths generally. That the patients have greatly benefited by treatment is beyond doubt, but it is too early to say anything about the ultimate results, though at least one such

patient treated over 5 years ago for recurrence after operation is still healthy and free from disease. In carrying out the treatment I endeavour to give as big a dose as the patient will stand, with the idea that if further treatment is necessary it should be repeated in two to four months' time. Patients seldom come up for a second treatment.

B.—Cancer of the Uterus, Uro-genital Tract, Abdominal and Thoracic Cavities (11 and 12).

Nine cases of cancer of the uterus were treated—one of the body and eight of the cervix. The technique for treating these cases has already been described. The brass tube usually employed has walls 2 mms. thick, and an external diameter of 10 mms. The tube is just long enough to contain the emanation capillaries, or radium cells—*i.e.*, the internal length is about 2 cms. In treating the body of the uterus, the tube is located in three positions, which do not overlap, two in the body and one in the cervix; and, finally, it is drawn into the aluminium distance piece tube, and treatment is given in the vagina. In cases of the cervix, usually the tube is first altogether in the cervix; secondly, half in the cervix and half in the vagina in the aluminium tube; and thirdly, in the vagina. The dose for each area is 6 to 9 mcd. When there is a large quantity of malignant tissue fungating into the vagina, radium needles are inserted where it can be done without risk of injury to neighbouring structures. Local treatment is usually supplemented by surface treatment over the pubis and parametria, and sometimes over the sacrum as well, a full dose being given with applicators 9 by 9 cms. As a result of this treatment hæmorrhage is practically always stopped, and often the discharge disappears. Occasionally, the discharge persists for some time owing to the disintegration of malignant tissues. In the case of cancer of the body of the uterus, inoperable on account of her heart condition, which was treated on 2nd May, 1925, for hæmorrhage, which had gone on for 11 months, a letter, dated 1st March, 1926, states that there had been no hæmorrhage since, but the patient was suffering some pain. In this case the patient received 25·0 mcd., divided up into three areas in the uterus, and a surface dose of 51·2 mcs. above the pubis and over the sacrum. Radium treatment seems to be satisfactory in carcinoma confined to the body—as one case, who received a similar treatment three years ago, has remained well since. A second remained well for a couple of years, but died from bronchitis, which condition originally prevented her being operated on, and was the reason radium treatment was tried for her. One of the cases of cervix (Case 2), in which the body of the uterus had been removed and part of the cervix, improved at first, but died after 10 months. In all the cases hæmorrhage ceased after treatment. One, treated five months ago (Case 1), has no discharge or hæmorrhage, is free from pain, and, so far, appears to have done well. One has improved considerably two months after treatment (Case 3), but has since been lost sight of. One, treated for a large fungating mass extending to the vagina in July, 1925 (Case 4), was seen in the beginning of this year complaining of continuous offensive discharge. Her general condition was much improved since treatment. On examination, the diseased area was ulcerated, but smooth, and not markedly indurated, giving one the idea of septic ulceration rather than malignant disease—a change which has been brought about by treatment for an undoubtedly malignant condition. When last seen the discharge was very much less, following the administration of 2 mcd. through a circular disc 20 mms. in diameter of ·5 mm. of brass, which was administered with the idea of improving the septic condition by producing a local

inflammatory reaction. Another patient (Case 5), who had been bed-ridden with pain and hæmorrhage for two months before treatment, was improved sufficiently to get about, and take solid food. She was lost sight of after leaving hospital, but probably succumbed to the disease. The sixth case was not treated in my own hospital, and no record has been received since. The seventh cervical case had a large ulcerating area extending down to within 2 cms. of the urethra, and a mass which projected into the bladder and rectum. Three months later the ulcerated area was healed, and completely covered by mucous membrane, and her bladder symptoms had disappeared. Although the local signs of disease were so much improved, the patient is losing flesh, and appears to be going down-hill. The last case, who had an extensive mass covered by mucous membrane surrounding the upper part of the vagina, though first relieved of pain, later complained of pain returning, and the growth broke down, leaving an ulcerated area. I believed there was a remote chance of this healing. Knowing I had given her a big dose, I advised against high-voltage X-ray treatment, which her friends arranged. She got a vesico-vaginal fistula in three days, and died a fortnight after this treatment, and three months after the radium. All these cases were inoperable, and the disease so far advanced that the parametria were already extensively involved when they came for treatment.

Cancer of Vagina.—Three cases of cancer of the vagina were treated. Two after excision of the malignant carbuncles of the external urethral opening, and one for malignant clitoris, in which radium was applied immediately after the growth had been removed by diathermy. The last case wrote nine months after treatment to say she was well. The two urethral cases have apparently been cured, although they suffered a good deal of pain for ten days or so a fortnight after the treatment. The treatment consisted in passing a tube into the urethra, and a couple of tubes applied on the surface opposite to it with 1 mm. brass screen and a dose of 1·5 med., combined with a surface application of 20 mms. distance and 9 by 9 cm. area and a dose of 20 med.

Cancer of Ovary.—One case of recurrent cancer of the right ovary, about the size of a child's head, which was rapidly growing, and causing great pain, was treated by surface application all over the tumour and in the right flank, using cross-fire as much as possible, and was given a dose of 273·5 med. at a distance of 15 mms. The emanation was so spread out that no ulceration of the skin was produced, though there was marked erythema. When the patient left hospital the tumour had shrunk very considerably, but, I understand, increased again after a couple of months, and she died a little over four months after treatment.

Cancer of Bladder.—Two very advanced cases of malignant disease of the bladder were treated by tubes in the bladder—one, with ten tubes placed systematically in different positions in the bladder through a supra-pubic opening. Both succumbed to the disease. That radium is some use in bladder cases I have proved, as a patient I treated three years ago by a tube in various positions in the bladder has remained perfectly well ever since. This case was inoperable at the time of treatment, and had been bleeding for months previously.

Cancer of Prostate.—One patient with cancer of the prostate was treated. He was 74, had heart disease, and passing a tube through the urethra or operative procedures of any kind were not considered advisable by the consultant who called me in. He was, therefore, treated with a tube in the rectum in three positions, dose 6·3 med.: over the sacrum, 16 med.; over the perineum,

from the sacrum to the coccyx, 66·3 med.; on the abdomen, 15 med.—a total of 103·6 mcs. at various distances from the growth. This had some effect in relieving distress, and stopped hæmorrhage for a short time, but could not be considered a radical procedure. He died from general asthenia six months later.

Abdominal Tumours.—That some effect can be produced by surface radiation alone in abdominal tumours is illustrated by the following case:—The patient was opened in the gall-bladder region for a tumour, which was found to be adherent to everything in the neighbourhood, so that the abdomen had to be closed without an attempt at resection of the mass. The patient suffered considerable pain, was unable to take her food, had frequent vomiting, and was obviously very ill. She was given a dose of 182 med., 46 of which was applied to the back of the abdomen opposite the growth, the remainder being applied in front over a 30-box area at a distance of 15 mms. When seen two months later she was up and about; she stated she had a good appetite, and could eat anything; she had no vomiting, and was able to sleep well. The loss of sleep had been a very distressing symptom. The tumour was much less distinct. I think it quite probable that improvement was temporary, but have not seen her since.

Mediastinal Tumours.—Two cases of mediastinal tumour were treated. In one, distressing cough and pain in the chest were relieved. The patient improved for a time, but died 4½ months after treatment. The dose in this case was 161·6 med., at 20 mms. distance. The other case got considerable relief from pain with a surface dose of 181·6 med., at 20 mms. distance; and, according to a recent report over three months after treatment, the improvement has continued.

C.—Cancer of the Mouth, Throat, Rectum, and Thyroid.

Twenty-four patients suffering from cancerous condition of the mouth were treated—comprising three of the floor of the mouth, one of the cheek, twelve of the tongue, two of the tongue and tonsil, and six of the tonsil. In last year's report (10), I fully recorded the technique employed in a case of cancer of the tongue. This case came up again last Spring, looking well, and a suspicious area of thickening at the junction of the tongue and anterior pillar of the fauces was radiated; dose, 1·7 med., area, 2 by 2 cm. through 1 mm. brass and 74·0 med. surface treatment through the floor of the mouth; and I have heard that the patient has remained well since; now 15 months. The general plan of treatment in a case involving the mouth is to insert at least six needles 7 cm. long into the diseased part through the skin under the jaw, when possible, without entering the septic oral cavity, and treating three different areas per needle by the method of withdrawing them definite distances, *i.e.*, 2 cms., described above. In the case of tonsils, the needles are inserted directly into them through the mouth. Where it is difficult to give an efficient dose by other means, I fix surface applicators against the ulcerated area by means of strips of aluminium sheeting, which are bent round the cheek and strapped to the surface, so that they stay exactly in the required position. Where there is ulceration underneath the tongue, two or more tubes, with thread or wire attached, are placed underneath the tongue, where they usually can be self-retained, the thread of wire being fixed by strapping outside on the cheek. In all cases the whole floor of the mouth, and at least the upper part of the sterno-mastoid muscle on each side, is intensely radiated by surface application, so that the malignant area is frequently radiated in three ways—by local application to the diseased area, by needles, and by surface application.

Of the three cases of floor of the mouth, one was lost sight of, the second has improved considerably, and the third, in whom the disease had been kept in check for two years, died.

The case in the inside of the cheek was treated by inserting needles through the cheek into the base of the ulcer, but not perforating the mouth, as well as surface application. This practically healed at one time, but has broken down again, and the result cannot be considered satisfactory.

Of the twelve patients treated with cancer of the tongue, one is apparently cured (Case 6), a second (Case 3) has some residual induration of the tongue and pain, but no very obvious signs of active disease ten months after treatment, and in another patient (Case 10) the result has been similar, five months after treatment. The disease in one patient (Case 2) was kept in check for two years and two months, for about a year appearing almost cured; he eventually died from septic pneumonia. Two very extensive cases (1 and 4) died within two months of treatment. One recent patient (Case 7) has done very well so far; and five patients (Cases 5, 8, 9, 11, 12) have been lost sight of. Immediately after treatment, all patients are able to talk much better, and are relieved from pain; but the disease is usually so advanced when they come for treatment, with extensive gland involvement, and their vitality is so low, it is impossible for them to respond sufficiently to treatment for much permanent good to be effected.

Of the two cases of tongue and tonsil, one, a man of 71, did very well, but died a few months later, after a couple of apoplectic seizures; the other patient was lost sight of.

One of the six cases of tonsil was practically healed up when last seen (Case 6). In another (Case 3), an enormous mass of post-operative glands in the neck almost completely disappeared for a time, recurred, and were again reduced by treatment; but he has since been lost sight of. The other four cases have not been seen since treatment.

One patient with cancer, the back of the arytenoid cartilage of the left side, was treated with a tube in three positions and surface application five months ago, and so far has done very well. Dose: against growth, 3·2 med. through 2 mm. brass, area 1 × 5 cm.; surface, 14·9 med.; distance 20 mm.; screen, 2 mms. brass; area, 2 × 6, opposite sides of the neck.

Three cases of cancer of the œsophagus were treated. One has been lost sight of; a second, who could only swallow liquids before treatment, was able to take turkey for his Christmas dinner, but has since died from extension of the disease and broncho-pneumonia. The third patient was still fairly well three months after treatment, when she was last heard of.

The technique in dealing with the œsophagus consists of getting down the largest tube that can be made to pass through the stricture, so as to radiate first the area below the growth; then the stricture area in one or two locations, according to the length of the growth in the gullet; and then the area above the stricture. The radium-bearing tube has screwed into it a fine Bowden wire, made up in sections 10 or 20 cms. long to the required length, and when in position does not cause much inconvenience to the patient, as the wire is very flexible. The free end of the wire projects through the mouth, where it is passed through the clipping apparatus devised for uterine work, which is strapped to the lips and cheek; and the whole arrangement is such that the exact position of the radium is known throughout the treatment, and it cannot be shifted during the treatment except by loosening the binding screw of the clipping apparatus. The tube usually employed is 7 mms. external diameter, with walls 2 mms. thick (brass), and a dose of 1 to 5 med. administered in each area. This is usually

combined with surface application, sometimes both at the front and back of the chest at the same time, in the areas directly opposite the site of the growth. I may mention that I have frequently found in cases where a tube could not be passed through the growth, that the surface treatment alone has decreased the dysphagia, and enabled the patient to swallow liquid nourishment more easily, for a shorter or longer period.

Four cases of cancer of the rectum were treated. One case had improved considerably by treatments in November, 1924, and February, 1925; and the doctor in the country told me he was doing fairly well, about nine months after the last treatment, when I last heard about him. One case was treated for a recurrence at the anal margin two years after operation, and appeared to be healing when last seen. One case was so advanced that he died ten days after treatment, and the fourth case has not been heard of since he left hospital.

The procedure in treating cancer of the rectum is similar to that employed in the œsophagus, except that a greater number of areas are treated well above and well below the growth—a dose of 2 to 3 med. is usually employed where the mucous membrane is apparently healthy, and up to 6 or 9 med. on the growth if it is massive. A tube 2 mms. brass walls, 15 mms. external diameter, usually increased to 20 mms. by the addition of rubber tubing, or a brass tube of 26 mms. external diameter covered by a finger stall, is used for the treatment. The exact position of the radium is regulated by the extension wire from the tube and the surface-clipping apparatus.

One case of cancer of the thyroid was treated, in which an attempt at removal had to be abandoned on account of extension of the disease. This growth had decreased greatly in size, and the operation wound appeared as if it was going to heal up when she left hospital to return to the country.

D.—Sarcomata.

During the year sixteen cases of conditions which were diagnosed as sarcoma were treated, but in several of these the diagnosis was not confirmed by microscopic examination. Nine of these patients suffered from extensive glandular involvement of the neck, which was adherent to everything and quite inoperable. In these cases the mouth and throat were examined by specialists, and no primary focus could be discovered to account for the condition. They were treated by a surface application of radium combined in most cases by the insertion of 6 needles or tubes, several areas being radiated by each of these in a manner already described. Six of these cases improved temporarily, some very markedly, but have not been heard of since they returned to the country. Three patients have since died. One of these, a man of over 70 years, was sent to me with glands in the right neck, principally because of the great amount of pain he was suffering. He was given a dose of 93·9 med. over 100 sq. cms. area, distance 15 mms., and when he left hospital there seemed to be no improvement. He came to me two months later to thank me, as his pain and the lump had completely disappeared. About a month later a small set of recent glands in the other side of the neck were treated, dose 43·7 med., 150 sq. cms. area, distance 15 mms. I saw him three months later, no glands were palpable, he was suffering no pain, but he was weak and debilitated, and obviously going down hill. There was no obvious cause for this condition except his age, and I heard he died exactly 9 months after treatment. Two cases of sarcoma of the humerus and one of the fore-arm were treated. One of the humerus cases was treated after removal of the head, and appears to be quite well, and is back at work. A second, who was in great pain and could

not move his arm without suffering agony, when seen 3 months later, was able to take off his coat and showed me his arm. Recently, 7 months after treatment, the arm is flail owing to the ununited fracture, the fore-arm is in good condition, he looks fairly well, but he is confined to the house with pains in the thigh and abdomen, possibly secondary deposits, though no tumour is obvious. Surface dose 89·2 med., 15 mm. distance 1·0 med. per sq. cm. was given. The case of the fore-arm, which was treated by surface application and the insertion of radium needles into the growth on two occasions, has now no pain, the induration at the side of the arm has considerably decreased, and he looks in good health. There is X-ray evidence of regeneration of bone, the radius at one time being apparently on the point of fracture. A case of sarcoma of the soft parts of the thigh treated after operation has been lost sight of. The growth in the infra-orbital region of an old man of 75 ceased to cause him trouble, and diminished greatly in size when he died suddenly from apoplexy nearly 4 months after treatment. One case of sarcoma of the naso-pharynx, who had become almost completely deaf owing to the extent of the growth, was treated by 6 radium needles, four through the right nostril and two through the palate into the naso-pharynx, dose 2·8 med.; also by a brass box 3 by 2 cms. internal measurements (surface box) and 3 mms. thickness of brass, inserted into the naso-pharynx, dose 3·6 med., and a surface application behind the jaw of 5·1 med. Three days later the patient's hearing was almost normal, and when the patient was examined by the specialist two months later no disease could be discovered, movements of the palate were normal, the holes in the palate had healed, and the deafness was completely gone, the patient being apparently cured. A somewhat similar case similarly treated two years ago is at present in hospital awaiting the fitting of an obturator. The sarcoma had perforated the palate when she was treated. She appears absolutely cured. The last case, a boy of 10 years, was apparently dying from suffocation, for which he was receiving inhalations of oxygen on the evening of the 18th April. He had a large mass of glands at the roots of the left neck, and the X-ray showed a tumour, which was principally on the left side of the upper part of the thorax, with marked narrowing of the trachea which was deflected to the left. He received a surface dose of 132 med. over the neck and both sides of the chest, front and back. On the 13th May, X-ray showed the trachea to be nearly straight and of normal calibre, and clinically the growth had diminished considerably. On the 3rd July he was given another treatment, as glands in the neck had increased slightly, dose 192·3 med. He went home, and was able to run about, though he had a constant slight rise in temperature. He returned to hospital in September, when I was asked to treat him again, as he was again suffering from some dyspnœa. As I was about to leave town, I arranged that my supply of radium, 53·93 mcs. in six 2 mm. brass tubes spread out over an area of 9 by 9 cm., at a distance of 15 mms. of felt, should be moved into different marked positions every three days over the front and back of his chest and neck, and the treatment should be continuous. The radium was on for sixteen days and 14½ hours. A dose 157·4 med. Unfortunately the children in the ward got chicken-pox, his attack being apparently most virulent. He died on the 10th November from this attack, over a month from the time the radium had been removed. At the post-mortem the mass of glands which remained was removed, and several sections examined histologically showed no evidence of Hodgkin's Disease or of sarcoma, only fibrous tissue being found.

E.—Rodent Ulcer.

Ten patients with rodent ulcers were treated, four of the eyelid, one of the ear, and five on the nose. Most of them have been lost sight of, as they came from the country. Three have been apparently cured by one treatment. I find beta rays very useful in the treatment of this condition, sometimes administered in serum needles with emanation capillaries, at other times by surface application. This is always combined with pure gamma radiation. Of two similar cases, both with small growths in the awkward angle between the nose and eye, one was treated by the radium cells, screen 0·2 mm. platinum and 1·25 mm. aluminium holder, area 7·5 × 15 mm., distance 13 mm. for 5¾ hours, 2·3 med., and through 2 mm. lead screen extra, for 5 hours, dose 2·0 med., total dose 4·3 med. for 10¾ hours; the other case was treated by 10 emanation capillaries containing 80·5 mc. at an average distance of 10 mm. without any screen for 8 minutes, through 0·5 mm. lead for 15 minutes, through 1·0 mm. lead for 17 minutes, through 1·5 mm. lead for 35 minutes, total 1¼ hours, dose 0·75 med. Both these cases healed with an inconspicuous scar. In the first case there was no marked reaction, and the local changes were complete in about three months. In the second case there was considerable redness and reaction less than 3 weeks after treatment, and the local changes were complete in about two months. These two treatments are interesting for comparison. In both the eye and side of nose were shielded by 3 mms. of lead.

Six cases of epithelioma of the skin of the face, six of the lower lip, and one of the nose were treated. One of the upper lip is apparently cured; a second of the lower lip was stated to be well four months after treatment. Two of the lip, who had been operated on three times previous to radiation, have since died, and the remainder were apparently on the way to being cured, and have been lost sight of. In one of these an extensive fungating growth on the lower lip, which bled to the touch, was curetted away two days after intensive beta-ray radiation, and was healing when he left hospital. All these cases received beta and gamma ray treatment on the same lines as the recent ulcer.

F.—Non-Malignant Cases.

Non-malignant cases are mostly treated by surface gamma radiation, sometimes combined with beta-ray treatment, for their superficial and local destructive effect on non-malignant growths, like papillomata. For the treatment of conditions when at all deep-seated a full erythema dose of 1·0 to 1·5 med. per sq. cm., at 15 to 20 mm. distance, gives the most satisfactory results. Much smaller doses are usually required for treatment of tuberculous conditions—for instance, tuberculous peritonitis (7) and pulmonary phthisis. A long, protracted dose with comparatively small radio-active material is specially indicated in lung cases, while a bigger dose may be given in tubercular peritonitis. I find it inadvisable to combine radiation with vaccine treatment when treating tuberculosis patients and those suffering from chronic bacterial infection.

Of the 88 non-malignant cases treated during the year, the following is a brief summary of the conditions dealt with, and results obtained:—

Hodgkin's Disease (1).—Glands practically disappeared on three occasions during two years' treatment. Patient ultimately died.

Asthma (1).—No obvious result from one short exposure.

Colitis (1).—This patient, aged four, suffered from mucous colitis for more than two months, and was in bed for six weeks. He did not appear to respond sufficiently to routine treatment, and would improve for a couple of days, and

then relapse, in spite of most careful dieting and medicinal treatment. As his cells appeared unable to deal with the infection alone, it was thought radium might help him. He was given a dose of 3·3 med. with a 9 by 9 cm. applicator on four areas in front of the abdominal wall, more particularly on the colon. In the next twelve hours the chronic condition appeared to be more acute, with griping, and more frequent stools. I considered this might be a reaction; and, as a certain amount of griping remained, I decided to repeat the treatment on the following day, using only half the dose. From that time the patient made an uninterrupted recovery, whether due to the very small radiation he received or not it is difficult to say; but, apparently, it did no harm. My own opinion is that the slight local reaction produced was sufficient to enable him to cope with the infection himself.

Conjunctivitis (1).—Immediate relief of pain followed. Subsequent history not known.

Corns (2).—Both corns disappeared.

Dupeytren Contraction (5).—All these patients were given full erythema doses, with distance pieces, as described above. One had been operated on, and had a recurrence, which had begun to interfere with the usefulness of his hand. He was treated first in May, 1924, and again in March, 1925, as a precautionary measure. The fibrous masses became smaller after the first treatment, and appeared to become inert. He is able to stretch his fingers fully, he feels no disability, and apparently there is no tendency to recurrence. A similar result was obtained in a case of a doctor who had not been operated on. Two other cases were lost sight of. Radium is very useful to prevent recurrence, which is very apt to take place after operation, even when a complete dissection is done; but it cannot be expected to relieve a case where the fingers are bound down to the palm by dense fibrous cords, where operation, combined with gamma-ray surface radiation, when the scars are soundly healed, give the best results, even better, in my experience, than using beta radiation by means of needles inserted in the tissues at the operation.

Fissure of Lip (1).—Healed in two days, and pain relieved at once, after ·029 med. 2-minutes β-ray treatment with 119 mcs. through 0·5 mm. glass.

Lumbago (2).—Surface radiation effected immediate improvement, and patients have remained free from further trouble to which they were subject.

Nævi (3).—Two cases on the nose and one on the lip were cured with inconspicuous scars—one with a total of ·66 med. through 0·2 mms. platinum, and 1·25 mms. aluminium.

Neuralgia (3).—One case of intercostal, one of dental, and one of anterior crural. Pain was relieved in each case.

Otorrhœa (1).—This patient was treated by a metal tube of 0·15 mm. thickness containing 207 mcs., for three minutes. A dose of ·077 med. As a result, the discharge increased for two or three days, completely stopped in ten days, and the patient states that her hearing has improved.

Papilloma, Rectum (1).—This growth, of malignant appearance, and nearly the size of a tangerine orange, had been ligatured, and when most of the growth had sloughed away radium was applied. Eight months afterwards the patient appeared to be perfectly recovered. Dose, 30·1 med. in two areas of the rectum through 2 mms. of brass, the tube 26 mms. external diameter.

Psoriasis (1).—Lost sight of.

Pruritus Ani (1).—Duration, 15 months. Followed by some immediate relief. Two days later increased discomfort, and subsequent gradual improvement, so that the patient three months later considers himself greatly benefited,

if not cured. Dose, 0·3 med. sq. cm. through 1·75 mms. brass, without distance pieces.

Scars (31).—Only two of these were treated for keloid, twenty-four following gunshot wounds received during the Great War, one for causalgia, two for painful scars following accidents, two as post-operative measure. The treatment of gunshot wounds has been carried on by me since 1917 (6).

These cases are only undertaken when continued treatment in the massage department ceased to improve the function of the part injured, or where experience shows that better and more permanent benefit will follow the treatment. This treatment undoubtedly loosens adherent scars, and enables stiff fingers to be moved more freely, both actively and passively; but it is necessary to continue re-educational exercises after radiation to obtain the full benefits of the treatment. One requires to stretch the adhesions when they are in a condition to be stretched. Radiation has repeatedly been found to relieve the tenderness of scars, and to enable patients with amputation to wear an artificial limb, when, before treatment, the stump was too tender to bear it. The nutrition of the limb is frequently improved by radiation of the scar in the neighbourhood of the big blood-vessels and nerves entering the limb, and trophic ulcers have healed up independent of weather conditions (8). One patient, with a painful scar on the sole of the foot, result of a burn seven years before, would have required an extensive operation for its removal. Such an operation was undesirable on the site. As a result of treatment, this scar became very painful about three weeks after treatment, remained so for a couple of weeks, but in two months' time a pliable smooth scar remained, which causes the patient no inconvenience.

A case of neuroma on the inner side of the knee, following a gunshot wound, which was exceedingly tender to the touch, excruciatingly painful when knocked by accident, and absolutely prevented the patient from riding a horse, was treated through 3 mms. brass-box, 3 by 2 cm. area, distance 15 mms. felt, dose 9·6 med. The next day the neuroma was excessively painful, and a rash developed, which accurately marked out the distribution of branches of the Long Saphenous nerve in the leg. The pain gradually disappeared, and in a week's time the neuroma could be squeezed between the fingers with impunity, and has caused him no inconvenience since. This patient was my resident at Steevens' Hospital when treated, and is still under constant observation, now for more than a year.

The following examples are from some of the cases of army pensioners radiated at Steevens' Hospital this year while on treatment at the Orthopædic Clinic, 51 Lower Mount Street, and further serve to illustrate the nature of the results obtained:—

1. G.S.W., Thigh. Crippled with sciatic pain since wounded, in 1914. Has had prolonged courses of treatment, including seven operations, two of which were on the sciatic nerve. Had had eight months diathermy treatment recently at Blackrock Pensions' Hospital, which only produced temporary relief. Suffers from marked twitching and tremor of leg, which became very pronounced when the sole of the foot was touched. Walked stiffly with the aid of sticks. Flexion of the knee limited. Stretching exercises and massages impossible. After radiation, which was applied for six days on scarred area of thigh, 59·6 med. gamma rays, patient could bear deep massage down the whole course of the sciatic nerve to the sole of the foot. Was able to flex and extend the knee well, though these movements continued to cause cramp in the calf muscles for a month after treatment, when the cramps disappeared. The patient at present has no cramps or tremors, is able to walk much further,

and has not to depend on a stick, and gets a good night's rest, which he was unable to do before.

2. G.S.W., Index Finger. Before treatment, metacarpo-phalangeal joint of first finger was very painful, and flexion of first and second fingers limited. After radium treatment, voluntary flexion of second finger greatly improved. Passive flexion normal; flexion first finger improved from 39 to 50. No pain. Grip improved from 9 to 19 during two months' massage treatment before radium, and then practically came to a standstill; and from 19 to 33 (33 normal for left hand) in less than two months after radium treatment. Is able to resume his work as a clerk.

3. G.S.W., Right Fore-arm. The arm was very painful, circulation bad, small ulcers on fingers, and hand very stiff. After radium, circulation improved, ulcers healed, scars are much more free, and have lost their redness, and become normal in appearance. No pain in the arm, and the hand is more supple. Girth of fore-arm increased ½ inch. Before radium, though on massage treatment, there was no appreciable muscular increase.

3. G.S.W., Left Shoulder. Complained of pain and drag in scar on raising arm; loss of sensation in left upper arm. After treatment the pain left the scar, and patient could raise the arm without discomfort; sensation returned to upper arm, and he was able to go to work.

5. G.S.W., Right Hand. Extensive scar back of thumb. Patient complained of pain and drag in scar, movement of fingers very poor, no grip registered by dynamometer. After treatment, scar became much softer, less painful, could be given deep massage, and in a short time his grip increased from 0 to 30.

6. G.S.W., Left Fore-arm. Painful scar ulnar side of fore-arm, fingers blue, no grip registerable. After treatment, scar appeared less tight, pain was better, and circulation improved. Grip increased to 5.

7. G.S.W., Left Fore-arm. Painful scar, and great drag on it, when third and fourth fingers were forcibly extended. Scar continually breaking down. Had two applications of radium within twelve months. The last time radium was applied he was so much better that he returned to work, and his wound has not broken down since.

I frequently have found that about one-third of a cancer surface dose hastens the healing of operative wounds, and renders the resulting scar less conspicuous, while with the full dose the scar in a recently stitched wound is liable to become stretched, and healing delayed. The incision for the removal of a cyst from the face in one case was treated for cosmetic reasons immediately after operation, and, similarly, another case was given a larger dose after the lengthening of the tendo achillis in order to hasten restoration of function, and so to minimise adhesion formation. In both these cases the desired result was obtained, apparently helped by radiation.

The two keloids treated appeared much improved when last seen. A full gamma ray dose is required in these cases, which sometimes has to be repeated.

Paronychia.—A case of chronic inflammation, causing redness round the root of a finger-nail, which resisted all treatment for two months, was found to be normal in appearance, and remained so, after a small amount of emanation, which had been strapped to the finger for four days, was removed. Dose, 0·75 mcd.; area, 2 × 0·5; screen, 1·0 mm., lead.

Tuberculosis.—Sixteen cases of various forms of tuberculosis were treated. The glands in three cases were smaller when last seen. In two cases of joint

disease a painful sinus at the angle healed up in one, and relief of pain followed treatment in the other.

One patient with a very chronic form of lung trouble, causing frequent hæmoptysis, has had treatment for the past four years—sometimes small quantities of radium, 1 milligram in each of two applicators, being applied for three to six months at a time. The tuberculous glands treated in his neck have completely disappeared. He had been in Steevens' Hospital 108 weeks before the first treatment with a temperature of 101 degrees level, and after one week of treatment the temperature became normal for the first time since admission, and remained so for more than a month. Dr. Winter, the physician in charge, expressed the opinion that this patient would have succumbed but for radium treatment.

Of two cases of lupus, one fairly extensive case of disease of the nose was cured after one treatment with a dose of 5·9 mcd., 0·7 mcd. with beta rays, and 5·2 mcd. gamma rays by surface application. In a case of extensive lupus of the face, about half the area treated is now replaced by a cosmetic scar, while the disease in the remaining parts appears much less deep-seated, and there is less induration. She is at present having another treatment for this with gamma rays alone. On the first occasion beta rays were employed, giving a dose of ·025 mcd. per sq. cm., and a total of 22·8 mcd. γ-ray treatment. This was followed by a good deal of reaction and pain. Six cases of tuberculous peritonitis were treated, one of whom was removed from hospital against advice, and has died. One of the cases has been opened, and the whole peritoneum was found studded with tubercule. A month after the operation, when treated, the abdomen was still distended and tender, and she vomited frequently. Three months after treatment she appeared perfectly well. Another, almost hopeless, case was given two treatments at two months' interval, and appeared to be quite well when last seen. One case is still in hospital with tuberculous hip; one was relieved of all pain and tenderness when last seen; and the sixth has been lost sight of. These cases are usually treated in six areas, 9 × 9 cm., over the abdomen (front) and an area over the spleen for five to seven days, at 15 mms. distance, and a screen of 0·2 mms. platinum and 2 mms. brass, a dose of 50 to 70 mcd. If patients are extremely ill, it is sometimes necessary to give less concentrated doses over a longer period. One such apparently hopeless case, who recovered, and has remained well for the last three years, had radium treatment for 54 days, and a dose of 58·9 mcd.

One tuberculous bone sinus and one ulcer of the foot were treated with small quantities of emanation.

Sepsis (2).—One case, with a sinus following the removal of septic glands, healed up rapidly, and the discharge from a septic tooth which had been present for six months ceased.

Septic Ulcers (8).—In all cases ulcer had been present for a long period. They are either known or are believed to have healed up.

When ulceration is very superficial, it is only necessary to use beta rays; but when induration is present, a good result cannot be expected without deep radiation as well. The first result of treatment in ulcers or sinuses is always a great increase of discharge, which is rapidly followed by its cessation. Provided the underlying area is healthy, beta rays stimulate epithelialisation in a truly remarkable manner.

Mooren's Ulcer (1).—One case of the cornea was relieved of pain following treatment, and the condition has improved somewhat, but was not nearly well when last seen. He requires further treatment.

Syphilitic Ulcers (2).—Ulcers in one patient had been healed in several

places, but the patient is at present under treatment for an extension in a neighbouring area. Another patient, with a specific ulcer in the leg, which was very indurated and dirty when first seen, was almost completely healed when he left hospital five weeks later with a pliable healthy scar. The latter patient had been seventeen weeks in another hospital, and received light treatment with a mercury vapour lamp. This, he states, did not give him instant relief from pain, and left a scar, which broke down as soon as he left hospital after the prolonged treatment. Undoubtedly, both forms of treatment do good; but the radium seems to be much quicker and more efficient, and leaves a more pliable scar.

Sprains (3).—One case in the back, one in the ankle, and one in the chest were treated. In the first case, the patient was subject to great pain from time to time, which confined him to bed. Since treatment, this has never recurred in this area, which had been constantly the source of his trouble. He got a similar condition, but not so severe, some distance above the original site, which was the first time it had ever varied its position. The pain and œdema of the ankle following injury in the second case disappeared after gamma ray radiation much quicker than one would expect. The pain in the chest in a third patient, due to strain in lifting, was similarly relieved.

Sciatica (2).—One was a doctor, more or less crippled by the condition for many years. There was immediate amelioration of the pain; and his son, who is also a doctor, told me some months later that the patient does not complain nearly as much as he used to. Dose, 28·8 med.; 72 hours; applicator, 9 by 9 cm.; two areas; distance, 15 mms.; gamma rays.

In the other case, who had to stay in bed on account of the severity of the attack, radiation appeared to alter the nature of the pain, but he was still very crippled with it for ten days, as I warned him he might be. I ventured to tell him that if he was completely free from pain in six weeks he might ascribe it to radium treatment. In less than six weeks he was practically well, and has now had no further trouble for more than a year.

MONOGRAPHS DESCRIBING TECHNIQUE, DOSAGE, AND APPARATUS USED BY THE WRITER.

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No. 27.

STUDIES ON PEAT. PART I. THE THERMAL DECOMPOSITION OF PEAT UNDER REDUCED PRESSURE.

By JOSEPH REILLY, M.A., D.Sc., F.R.C.Sc.I., F.I.C.,
AND
GERALD PYNE, B.Sc. (Lond.), A.R.C.Sc.I., F.I.C.,
both of University College, Cork.

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Introduction.

The method of rapid distillation under reduced pressure developed by A. Pictet and his collaborators has, in recent years, been applied with considerable success to the investigation of various types of organic matter, such as cellulose and cellulosic products, starch, various glucosides, inulin, gelatin, albumins, oils, &c. (Helv. Chim. Acta, 1918, **1**, 87; 1919, **2**, 188, 501, 698; 1921, **4**, 616; Comptes rend., 1913, **157**, 779, 1436; 1917, **165**, 113.) The success obtained in this type of distillation with minimum decomposition of intermediate products is largely due to two causes—(*a*) the process takes place at a much lower temperature than when the distillation is carried out at ordinary pressure; (*b*) the greater portion of the products of decomposition is quickly removed from the sphere of decomposition, and, in consequence, the occurrence of secondary reactions, which tend to reduce the amounts of the intermediate products, is, to some extent, prevented.

The results obtained from cellulose by the treatment are of considerable interest. On decomposition by heating under reduced pressure, Pictet obtained a first distillate (45 per cent.), from which a well-defined crystalline product, *l*-glucosan, was isolated. This substance is an anhydride of glucose, $C_6H_{10}O_5$, which it reforms readily on boiling with dilute acid. It is neutral, very soluble in water, and does not reduce Fehling's solution. By the action of benzoyl chloride and alkali it forms a tri-benzoyl derivative, showing thus the presence of three hydroxyl groups. This reaction is usually employed for its isolation from the distillates of various products which yield it, when they give rise to small amounts only (*e.g.*, some sugars). The dry distillation of cellulose at ordinary pressures, on the contrary, is much more complete, and gives rise to quite a different set of substances, such as acetic acid, acetone, and tar, containing, among other things, complex mixtures of hydrocarbons, furfurane derivatives, and phenols. In addition, both types of distillation give rise to considerable quantities of gases.

The vacuum distillation of coal has been studied by Pictet and Bouvier (Compt. rend., 1913, **157**, 779–81), and by Wheeler and Jones (J.C.S., 1914, **105**, 140). The results are somewhat conflicting. The former workers, using rapid distillation at a temperature up to 480° C., obtained about 4 per cent. of tar. No phenols were present in their distillates, nor was ammonia or solid paraffin detected, but appreciable quantities of menthol-like compounds and of bases, chiefly secondary, were obtained. Wheeler and Jones, on the

other hand, obtained, besides gaseous products, about 6·5 per cent. of a tar, about half of which on distillation remained as pitch; while the other half consisted mainly of naphthenes and unsaturated hydrocarbons, with smaller amounts of phenols, paraffin, and aromatic compounds. Their distillations were carried out slowly—occupying several weeks—and to this fact, as well as to differences in the types of coal used by the two investigators, are attributed the very different results. It may be remarked that the general type of product obtained by Wheeler and Jones is like that obtained by the distillation of coal at ordinary pressures, and is thus in striking contrast to the results obtained by Pictet for cellulose and similar materials.

In view of these facts, it seemed desirable to try the effect of vacuum distillation on peat, a substance intermediate in properties and structure between the largely cellulosic plant residues from which it arises, and the coal into which it eventually passes. No previous work seems to have been carried out on these lines with dry peat, although its distillation under ordinary pressure has been fairly thoroughly investigated, as well as low temperature distillation in steam. In the ordinary distillation, the following substances are obtained:—

(1) *Coke, or charcoal,* consisting of the fixed carbon and the ash or incombustible mineral matter.

(2) *Tar,* a mixture of various hydrocarbons, and phenolic compounds, formed by the destruction of the complex organic substances in the peat.

(3) *Tar-water,* a solution of a number of the lighter, less easily condensed organic and other compounds in the water originally present in the peat or formed by its decomposition.

(4) *Fixed gases*—these are similar to those obtained from coal, and can be used for illumination. The yield varies considerably for different kinds of peat; the less decomposed giving the largest quantity and poorest quality.

From the tar, which rarely exceeds 4·5 per cent. of the dried peat, light petroleum, lubricating oil, wax, creosote, and asphalt can be obtained; and from the tar-water, acetic acid, methyl alcohol, ammonia, and other bases, such as pyridine, aniline, &c. The composition of the tar-water varies with the nature of the peat from which it has been formed—fibrous peat giving relatively large quantities of acetic acid and methyl alcohol, and dense lowland peat larger quantities of ammonia and other bases.

Thenius, quoted by Ryan (Econ. Proc. Roy. Dublin Soc., 1908, **1**, p. 516), obtained from good air-dried peat the following amounts of by-products:—

Substance.	per cent.	
Charcoal	35·34	
Tar water	40·00	
Light petroleum ...	1·76	
Heavy petroleum ...	2·56	
Lubricating oil ...	1·77	9·03
Wax	0·30	
Asphalt	1·56	
Creosote	1·08	
Gas and loss	15·63	

From other figures given for the composition of the tar water, it can be calculated that the following yields were obtained in terms of the air-dried peat:—

	per cent.
Ammonium sulphate ...	1·60
Methyl alcohol ...	0·80
Pyridine bases ...	0·08
Acetic acid	0·63

These figures apparently refer to a remarkably good type of peat, as the amount of tar here recorded, 9·03 per cent., is about twice that usually obtained;

it represents, however, fairly well the type of material employed in our experiments.

The peat used in the work about to be described was a heavy, good-quality sample, taken from the second spit, *i.e.*, from the sod 9″ to 18″ beneath the surface. It was obtained from a working bank midway between Drimoleague and Bantry, in County Cork, by Mr. Duffy, A.R.C.Sc.I., to whom the authors wish to express their thanks. In the raw state it contained 85 per cent. of water. For experimental work, it was air-dried, and was then friable enough to grind. The powder was dried to constant weight in the water oven, and used in the anhydrous state for all subsequent work. The anhydrous peat contained 1·51 per cent. of nitrogen, and 5·30 per cent. of ash.

One of two preliminary vacuum distillations carried out with the object of ascertaining the general type of products formed, seemed to indicate that the decomposition followed, more or less, the same lines as a distillation at ordinary pressure, apparently resembling in this behaviour coal, rather than cellulose. It was accordingly decided that most information would be obtained by running two distillations with the peat—one under reduced pressure, and the other under normal conditions, and comparing the amounts of the different substances formed in each. Attention was, therefore, directed mainly to determining the amounts of products—such an ammonia, acetic acid, methyl alcohol, phenols, resins, wax, &c.—recoverable from the distillates, and to the composition of the gases liberated.

Experimental.

The experimental work which will now be described may be divided into three main parts:—

(A) Comparative low pressure and ordinary pressure distillations at moderate temperatures.

(B) Comparative distillations at higher temperatures.

(C) Comparative distillations of small quantities to investigate the gases formed.

Before proceeding to take these in detail, it will be necessary to give a brief account of the method employed in carrying out the low pressure distillation, and of the analytical methods used to estimate the various constituents in the distillates.

Vacuum distillation.—The apparatus employed for the distillation was a 400 c.c. glass distilling flask, to which was fixed a smaller flask, which was kept at a temperature of about 60° C., so as to retain any tar which came over, and, as far as possible, avoid any condensation of the aqueous portion of the distillate therein. To the flask was attached another, surrounded by a freezing mixture of ice and salt, to condense water and other volatile products. Between this vessel and the water pump a bubbler containing dilute sulphuric acid was introduced, to retain any ammonia which might be swept out by the evolved gases, and to indicate the rate of the reaction. Corks were used for closing and connecting the distillation flask, and were made air-tight by a paste of sodium silicate. The whole system was exhausted; and while the pump was still running, the distillation flask was plunged into a bath of a molten mixture, of equal parts of sodium and potassium nitrates, which could be heated by a fairly large Teclu burner. The heating of the bath was adjusted so as to obtain a fairly regular stream of gas through the bubbler, but not so rapid as to cause any great change of pressure in the system. Distillation was continued

until the gas stream became slow, and tar ceased to come over. Usually, 4 to 8 hours sufficed for this. The distilling flasks and the two receivers were weighed before and after the distillation to determine the weights and percentages of the different fractions.

The procedure in the ordinary pressure distillations was the same, except, of course, that the water pump was cut out. A sharp separation between tar and aqueous distillate is not, however, brought about simply by keeping the first receiver at 60° C. The tar usually contains a fair amount of admixed water, and a small amount of tar is carried over into the second receiver. In order to estimate the tar obtained, both receivers are drained of their water, washed with a small amount of hot dilute sulphuric acid, then cooled to solidify the tar, and the acid poured off, and added to the water previously removed. The washing is repeated with a small quantity of hot water, and the residue of tars dried and weighed. By subtracting the weight of tar thus obtained from the sum of the weights of the distillates in the two receivers, the quantity of aqueous distillate is obtained. The amount of tar was, as a rule, too small for separation by fractional distillation, but was worked up for phenols, resins, and wax. The combined aqueous distillate and washings were added to the contents of the bubbler, and used for the determination of methyl alcohol, acetic acid, and ammonia.

Treatment of aqueous distillate.—This was first made alkaline with sodium hydroxide solution, and distilled. *Ammonia* was determined by receiving the distillate in standard acid, and titrating. *Methyl alcohol* was estimated by redistilling the ammonia distillate from acid and carefully determining the density of the distillate. The residue remaining in the flask from the ammonia distillation was worked up for acetic acid, as will be described.

Acetic acid was determined in the residue from the ammonia distillation as follows:—Excess of barium hydroxide was added to remove any carbonate and sulphuric acid, which, in the subsequent distillation with phosphoric acid, might give rise to carbon dioxide by its action on organic matter. The solution was filtered, concentrated, re-filtered into a glass retort, and distilled to incipient dryness with 3 to 4 grams of syrupy phosphoric acid. After the distillation the residue was redissolved in the retort by the addition of about 50 c.c. of hot water, and distilled again almost to dryness. This was repeated three or four times, and the combined distillates titrated with N/10 sodium hydroxide solution, using phenolphthalein as an indicator. Acetic acid from the first vacuum distillation was not estimated.

Treatment of Tar.—Estimations were made of phenols, resinous substances, and wax by the following methods:—

Phenols.—The tar was extracted three or four times with moderately dilute sodium hydroxide in a separating funnel, to which a fair amount of ether had been added to facilitate the separation. The combined alkaline extracts were acidified with dilute sulphuric acid, and distilled in a current of steam till about 600–700 c.c. of distillate was collected. This was made alkaline with sodium hydroxide solution to prevent loss of phenol by volatilisation, evaporated almost to dryness, and washed into a small separating funnel. Dilute sulphuric acid was added to liberate the phenols. (Attempts were at first made to do this in a burette, so as to read off directly the volume of phenols liberated, but the evolution of carbon dioxide which occurred made it difficult to manage.) The aqueous layer was run off, the remaining phenols dissolved in a few c.c.s of alkali, transferred to a narrow burette, liberated by dilute sulphuric acid, and measured directly. (Immersing the burette in a

large cylinder of hot water facilitated the separation.) It was sometimes very difficult to read off the volume of phenols, as they were semi-solid, and inclined to lump together. In such cases they were dissolved in petroleum ether (B.P., 70° C.), and shaken with a known volume of a solution of alkali. The increase in volume of the alkali was a measure of the phenols.

Resins.—A black sticky mass of resin remained in the flask from which the phenols had been steam-distilled. To get this into filterable condition, it was dissolved by addition of alkali, and reprecipitated by dilute acid, when it was obtained in flocculent form, and could be filtered off, washed, dried, and weighed.

Wax.—The tar remaining after extraction of phenols (and partly dissolved in ether) was transferred to a porcelain basin, and evaporated to dryness. It was then treated with successive portions of hot alcohol, and these filtered through a hot-water funnel. The filtrate on cooling solidified to a gelatinous light-brown mass. A small amount of substance insoluble in hot alcohol remained—possibly resinification products, formed from the tar by the action of alkali. The alcoholic solution was evaporated to dryness (and in later experiments the residue weighed, to determine alcohol-soluble tar), and dissolved, as far as possible, in a mixture of equal parts of ethyl alcohol and ether. A considerable amount remained insoluble. The dark solution obtained in this way was cooled to −20° C., and filtered under pressure through a small filter paper and funnel, surrounded by a freezing mixture of ice and salt. On cooling the solution in this fashion, paraffins separated out, and were filtered off. Attempts were made at first to cool the ether-alcohol solution to −20° C. by means of ice and salt, but this proved unsuccessful, and subsequently solid carbon dioxide and ether were used. The paraffin on the filter paper was washed with small quantities of ether-alcohol mixture, cooled to −20° C., and then washed through the filter paper with hot petroleum ether (B.P., 70° C.) into a weighed basin. This was then dried and weighed.

A.—*Comparative Distillations at Moderate Temperatures.*

(1) *Low pressure distillation.*—A general account of the method of working has been given already. The following are the details of an actual distillation:—

86 grams of peat was introduced into the distillation flask, which was then exhausted to a pressure of about 10 mm., and lowered into the nitrate bath at 360° C. A very rapid evolution of gas took place, and the pressure rose quickly to about 50 mm., and during the next quarter of an hour nearly reached 100 mm. The burner was removed for a while, until the pressure fell again to about 50 mm., and was then replaced, and the heating regulated so as to prevent too vigorous evolution of gas, with consequent rise of pressure. By about one hour after the experiment began the pressure had fallen to 20 mm., and during the remaining three hours the experiment lasted it continued to fall slightly. From the moment heat was applied, white vapours began to pour over into the receivers, and after a few minutes a light yellow-coloured wax-like tar began to collect in the first receiver. The internal temperature (taken in the neck of the distilling flask) was considerably less than that of the bath. It reached 200° C. after half an hour, and remained almost constant at that figure throughout the distillation. The tar which collected in the first receiver was a semi-solid light-brown waxy mass, unlike the dark, viscous liquid tars obtained by ordinary distillation. A considerable amount of light, powdery

coke remained in the distilling flask. The percentage weights of the different fractions are shown in Table I, column A (i).

TABLE I.

	A (i). per cent.	A (ii). per cent.	B (i). per cent.	B (ii). per cent.
Viscous distillate collected at 60° C. ...	13·64	32·27	14·59	31·46
Aqueous distillate in cooled receiver ...	12·64	0·99	14·92	0·65
Residue in flask (coke) ...	59·67	51·41	48·14	44·42
Gas and loss	14·05	15·33	22·35	23·47

On separating the tar and tar water in the manner already described, the figures shown in Table II, column A (i), were obtained.

TABLE II.

	Low temperature. A (i). per cent.	Low temperature. A (ii). per cent.	Higher temperature. B (i). per cent.	Higher temperature. B (ii). per cent.
Tar	10·18	9·50	17·20	15·61
Tar-water	16·10	23·76	12·31	16·50
Coke	59·67	51·41	48·14	44·42
Gas and loss	14·05	15·33	22·35	23·47

Analyses of the tar and aqueous distillate were carried out by the methods indicated earlier. The results are shown in Table IV, column A (i).

These results will be discussed later in comparison with those obtained by distillation at ordinary pressures. Two constituents, paraffin and phenols, will, however, be considered separately now.

From the appearance of the distillate which came over in the earlier stages of the heating—light yellow and wax-like—it was at first thought that some of the so-called peat waxes—substances resembling Montana wax—were distilling unchanged. Morgan and Scharff (Econ. Proc. Roy. Dublin Soc., 1915, **2**, p. 161) examined a sample of peat tar from a gas producer in Portadown, and separated a wax which they considered to resemble the Montana wax investigated by Ryan and Dillon (Scien. Proc. Roy. Dublin Soc., 1909, **12**, p. 202). In view of these points, the wax obtained from the vacuum distillation was examined to see if it showed any evidence of being an ester, or wax properly so-called, or an acid, such as Montanic acid.

The saponification value of the wax was determined, and found to be zero; so that it appears from this, and from the method used for its isolation, to be a paraffin (although the possibility of its being a higher alcohol is not excluded, and might require a combustion to settle). The wax is, however, in all probability, a pyrogenic product, unconnected with the waxes which can be extracted from peat by solvents. This point is being further investigated.

Morgan and Scharff also examined the phenolic substances contained in the Portadown peat tar, and showed that the higher boiling fractions were much more highly germicidal than phenol and its homologues, and were distinguished from them by giving different colorations with 4-triazo-3 : 5-dimethylpyrazole in alkaline solution, a reagent which had been shown by Morgan and Reilly (J.C.S., 1914, **105**, p. 442) to develop characteristic colorations with aromatic

hydroxyl derivatives. (The phenols of ordinary peat tar have been carefully studied by Bornstein and Bernstein (Zeit. f. angew. Chemie, 1914, 27, p. 71), who showed the presence of phenol, all three cresols, 1.3.2. and 1.2.4. xylenols, and the mono-methyl ethers of catechol, homocatechol, and pyrogallol.) As the amount of phenols obtained here was very small (less than 0·5 grams), no systematic examination could be attempted; but an attempt was made to determine whether the phenols obtained by vacuum distillation gave different colorations with 4-triazo-3 : 5-dimethylpyrazole from those obtained by ordinary distillation. The phenols were dissolved in ether, dried over anhydrous sodium sulphate, and, after removing the ether, distilled under a vacuum in a very small distilling flask. Two small fractions, which came over about 70° C., were collected, and, with the residue in the flask, were tested with the above reagent.

TABLE III.

Phenolic substances from peat.	Reaction with 4-triazo-3 : 5-dimethylpyrazole in alkaline solution.	
	Coloration after a half-hour.	Coloration on long standing.
First Fraction ...	Deep violet.	Reddish brown.
Second Fraction ...	„ „	„ „
Residue ...	Greyish blue.	Brownish.
	Coloration.	
Phenol	Purple, changing to deep azure-blue and finally to brown.	
o-cresol	Bluish violet, redder than the preceding coloration, changing to dark red.	
m-cresol	Intense blue, changing slowly to brown.	
m-4-xylenol ...	Faint bluish-violet, changing to light red.	
o-xylenol	Mixed red and blue, changing to light purple.	

Table III shows the colorations obtained. The colours given by some of the commoner aromatic hydroxy-derivatives are appended for comparison (cf. Morgan and Reilly, *ibid*). On account of the small amount of substance available, the separations between the fractions are very indefinite; on the whole, however, the phenolic substances present appear to contain some cresols; more than this cannot be said. It may be mentioned here that the phenolic substances obtained from the distillation of the same peat under ordinary pressures gave somewhat similar colorations. Apparently, such essentially pyrogenic substances as phenols do not serve to bring out the differences between the two modes of distillation.

(ii) *Distillation under ordinary pressure.*—This was conducted with 62·4 grams of dried peat, in the same type of apparatus as was used for the vacuum distillation, except that the system, instead of being attached to the water pump, was open to the air. The flask containing the peat was lowered into the bath of molten nitrates, which soon reached a temperature of 350° C. The internal temperature in the neck of the distilling flask was then 100° C., and remained so for nearly an hour, much water coming over meanwhile. On increasing the heating, the internal temperature rose to 160° C., and after a while to 200° C., and finally reached 235° C. Meanwhile a dark brownish-black fluid tar was coming over, differing considerably in appearance from the vacuum tar. After about four hours' heating, the temperature of the bath was raised as high as possible with the burner employed, and kept so for another four

hours, the distillation occupying eight hours in all. Very little more distilled during this period. The internal temperature remained at about 270° C.

The greater part of the distillate, tarry and aqueous, was contained in the first receiver, kept at 60° C., only a small amount of tarry material having got as far as the second. (See Table I, column A (ii.)

The tar and water were separated in the manner described already, and weighed. The percentage weights are shown in Table II, column A (ii).

Analyses of the tar and aqueous distillate were carried out as before. The results are shown in Table IV, column A (ii).

TABLE IV.

	Low temperature.		Higher temperature.	
	A (i). per cent.	A (ii). per cent.	B (i). per cent.	B (ii). per cent.
Ammonium sulphate ...	0·35	0·44	0·76	0·94
Methyl alcohol	0·32	0·34	0·30	0·37
Acetic acid	—	—	0·55	0·84
Phenols	0·52	0·32	0·64	0·64
Resins	1·13	1·27	3·71	1·22
Paraffin	0·45	0·22	0·87	0·39

From these figures in Tables II and III it can be seen that, although the decomposition of the peat is more complete than in the vacuum distillation, as judged by the percentage of coke remaining, the yield of tar is actually less. The aqueous distillate appears greater, however, but exactly how far is more difficult to say, as a large part of the water which distils over in the vacuum work is sucked down the pump, and is returned under the head of gases and loss. In spite of this, however, "gases and loss" are higher in the ordinary distillation. It is probable then that not only is the formation of tar greater in the vacuum than in the ordinary distillation under identical heating conditions, but that also the formation of gas is less. To some extent, the larger formation of gas in the ordinary distillation may be due to the presence of air, with consequent oxidation; but experiments, to be described later, in which air was not allowed access to the peat, show the same result., *i.e.*, smaller gas formation in vacuum distillation than in ordinary pressure distillation under the same conditions. All this points to a type of decomposition under very reduced pressures, which leads to increased production of the heavier and more complex products, and decreased production of the more volatile. The figures for particular constituents bring out in more detail what has been already noted about the type of decomposition. Ammonia and methyl alcohol are formed in larger amounts in the ordinary distillation than in the vacuum, although the ammonia in both cases is low. This is probably accounted for by the anhydrous condition of the peat used. It is recognised that, for maximum production of ammonia, peat must contain a fair amount of moisture—steam is often blown through the heated peat for this purpose—so that the conditions in these experiments were very unfavourable to the formation of ammonia. On the other hand, the amount of solid paraffin formed by vacuum distillation is more than twice that obtained by ordinary pressure distillation. Phenols appear to be greater also; but, as these were only distinguished from the resins by their volatility in steam, it might perhaps be better to take the sum of resins and phenols for comparison. The sum is slightly greater in the vacuum distillation.

B.—*Comparative Distillations at Higher Temperatures.*

The considerable residues of coke left over in the distillations, both vacuum and ordinary, just described, seemed to indicate that the decomposition had not been pushed as far as possible, in spite of the long heating. It was, therefore, decided to repeat the work at a higher temperature.

(1) *Low pressure distillation.*—This time the flask containing the peat (55·0 grams) could be almost totally immersed in an iron pot containing the nitrate mixture, heated by a very large Meker burner. A much higher temperature could be reached by these means. Two water pumps were used to take off the gases evolved, so as to prevent the pressure becoming too high. The distillation was begun by immersing the flask in the nitrate bath at 300° C., which was gradually heated as strongly as possible. The pressure was at first 12 mm. During the first hour the evolution of gas was very rapid—distillation was apparently complete in four hours. The weights of the various fractions are shown in Table I, column B (i).

Tar and water, which were somewhat admixed in the two receivers, were separated and weighed. (See Table II, column B (i).) The detailed analyses will be considered later.

(ii) *Ordinary distillation*: *High temperature.*—A distillation was also carried out under conditions more nearly approaching those in technical practice. A copper flask was used to hold the peat, and was heated directly by the Bunsen flame. An asbestos stopper, made air-tight with a paste of sodium silicate, was employed. The rest of the apparatus was as usual. The peat used weighed 62 grams. The heating had to be carefully regulated at the beginning to prevent the production of dense white fumes, which passed through both receivers uncondensed. By the end of three hours, distillation appeared to be complete. The percentage weights of the fractions obtained are shown in Table I, column B (ii).

Nearly all the tar and tar-water condensed in the first receiver. These were separated and weighed. (See Table II, column B (ii).)

The very much higher temperatures reached in these distillations have decreased the residue of coke, and increased the yield of tar. The latter is still higher in the vacuum distillation, however, notwithstanding the stronger heating received by the peat in the copper flask during the distillation at ordinary pressure. The tars and aqueous distillates obtained in this and the previous (vacuum) distillation were worked up for paraffin, ammonia, acetic acid, &c. The results are given in Table IV; where column B (i) refers to the vacuum distillation, and B (ii) to that carried out at ordinary pressure.

It can be seen that the more vigorous heating has caused considerable increases in many of the constituents; ammonia and paraffin are about twice as high as in the corresponding distillations carried out at lower temperatures. Methyl alcohol remains much the same. The yields of the simpler products, such as ammonia, methyl alcohol, and acetic acid, are greater again in the ordinary than in the vacuum distillations; the yields of the more complex, such as wax, are less. Phenols do not show much difference, but there is much more of the heavier and less volatile acidic substances—described as resins—in the vacuum distillate. These results confirm what had been noticed in the earlier and less complete distillations—namely, the increased production of the heavier and more complex products, and lessened production of the more volatile, in distillations carried out under reduced pressure. This effect shows itself in the different amounts of gas produced in the two types of distillation, as will now be seen.

C.—*Comparative Distillations of Small Quantities for Examination of the Gases Evolved.*

1-gram samples of peat were distilled under both atmospheric and reduced pressures, and the gases collected and examined.

(i) *Vacuum distillation.*—The distillations were carried out in a small distilling flask, A, sealed to two small glass bulbs, which acted as receivers; the first, B, being kept at 60° C. by a water bath, and the second, C, surrounded by a freezing mixture. The second receiver was connected with a Sprengel pump, D, by the use of which the evolved gases could be collected over mercury in the gas tube, E (see figure). The whole system was exhausted, and while the pump was still working the distilling flask was lowered into a bath of molten mixed nitrates of sodium and potassium at 350° C. The source of heat had to be removed for a while, as the evolution of gas was too rapid for the Sprengel to deal with, and the pressure rose in five or six minutes to 100 mm. In about fifteen minutes it had returned to a few millimetres, when the heating was increased. Gas then evolved steadily for nearly an hour. After that the rate of evolution slackened considerably, but did not become negligible until about four hours later. During the first five minutes about 30 c.c. collected, during the next fifteen minutes about 20 c.c., and during the rest of the distillation period something over 60 c.c. The gases were collected in two receivers—the first 80 c.c. in one, and the last 30 c.c., or so, in a second vessel. These were analysed separately to get some idea of the variations in their composition during the course of the distillation. Table V gives the results.

TABLE V.

	Fraction I.	Fraction II.	Total gas.
Volume. N.T.P.	80·0 c.c.	32·2 c.c.	112·2
	per cent. by vol.	per cent. by vol.	per cent. by vol.
Carbon dioxide	62·6	16·0	49·2
Carbon monoxide	20·1	16·0	18·9
Unsaturated hydrocarbons ...	3·6	2·3	3·3
Saturated gases	13·7	65·7	28·6

The total volume of gas evolved was 112·2 c.c. at N.T.P. The principal change during the distillation is the very considerable fall in carbon dioxide, accompanied by a corresponding increase in saturated gases. Carbon monoxide remained fairly constant in amount, showing only a slight fall.

(ii) *Ordinary distillation.* — The gases evolved from 1 gram of dry peat powder on distillation at ordinary pressure were also collected and analysed. The same type of apparatus was used as for the vacuum distillation, except that the Sprengel pump was not operated until the end of the experiment, and was then used to collect gases remaining in the distillation flask and receivers. To prevent oxidation of the peat, which the presence of air might have brought about, the system was filled with carbon dioxide before heating. The volume of carbon dioxide needed was not great, as the capacity of the distilling flask and attached receivers was small, and it was assumed that all the carbon dioxide thus added was swept out early in the evolution of gas. The volume of carbon dioxide originally present was determined from the capacity of the flasks used, and subtracted from the volume of gases collected. The gases were collected in two fractions and analysed separately. Table VI gives the results.

TABLE VI.

	Fraction I.	Fraction II.	Total gas.
Volume at N.T.P.	85·0 c.c.	57·8	142·8.
	per cent. by vol.	per cent. by vol.	per cent. by vol.
Carbon dioxide	64·8	21·7	47·3
Carbon monoxide	12·3	10·8	11·7
Unsaturated hydrocarbons ...	3·0	2·8	3·0
Saturated gases	19·9	64·7	38·0

There are some points of difference between these figures and those obtained by vacuum distillation. Carbon monoxide is uniformly less here, and saturated hydrocarbons are present in larger amounts. Unsaturated gases are not present to quite the same extent as in the vacuum gases. Furthermore, the total volume of gas is considerably greater, viz., 142·8 c.c. instead of 112·2 c.c. Fractions I and II illustrate the same falling off in carbon dioxide and corresponding increase in saturated hydrocarbons during the distillation and the fair constancy of the carbon monoxide content throughout. Fraction II is not, however, quite comparable with the Fraction II obtained under reduced pressure, as it forms a larger percentage of the whole gas evolved.

The difference in composition between the gases obtained by the two methods of distillation is not, however, as marked as the difference in volume. The volume of gas obtained by the ordinary distillation is very much the greater. This is in agreement with what has been noticed in comparing the other constituents, i.e., the vacuum distillation gives in general a less complete decomposition than the ordinary; intermediate substances are formed in greater amounts, and simpler products, such as gases, in smaller amounts.

Examination for distinctive products of vacuum distillation.

The vacuum distillation of cellulose gives rise, as has been mentioned already, to a sugar *l*-glucosan, and various furfural derivatives. An attempt was made to test for the presence of these substances in the distillate from peat, which on account of its origin might contain appreciable amounts of cellulosic material. A vacuum distillation was carried out in the manner described, using about 20 grams of peat. The tarry distillate was extracted with hot water, cooled, filtered, and the filtrate tested for furfural with aniline acetate. A fairly strong coloration was obtained. The filtrate was then exhaustively extracted with ether to remove furfural bodies, and the following tests carried out on the aqueous part:—

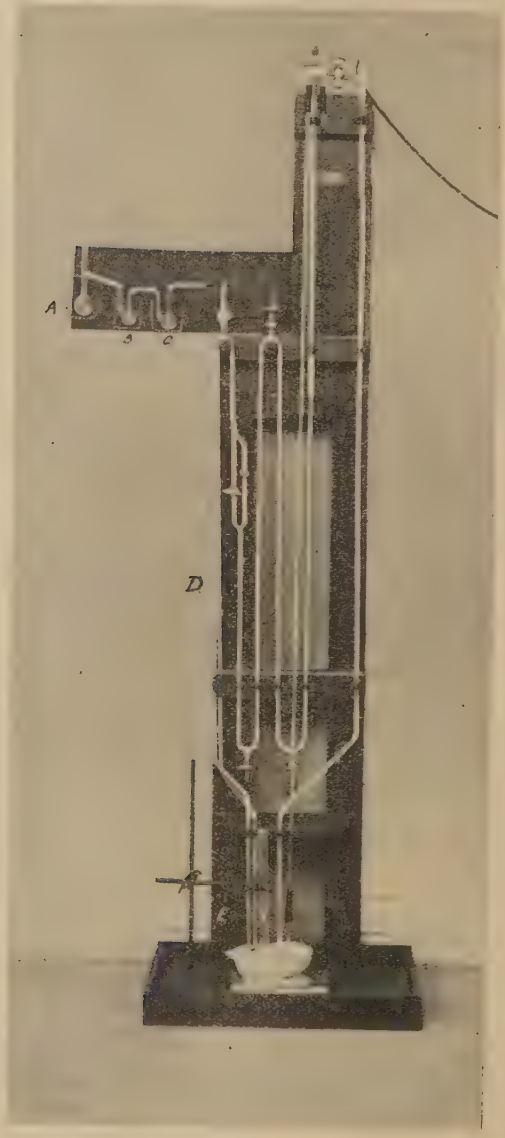
(I) Fehling's solution only very slightly reduced. On boiling the aqueous extract for a few minutes with dilute sulphuric acid, making alkaline, adding Fehling's solution, and boiling, a heavy precipitate of cuprous oxide was obtained.

(II) Ammoniacal silver nitrate was immediately reduced without formation of a mirror.

(III) Alkaline permanganate was immediately reduced.

(IV) Addition of benzoyl chloride and dilute sodium hydroxide solution followed by vigorous shaking gave a very small amount of a solid benzoyl derivative which was not further investigated.

(V) Part of the aqueous extract was refluxed for some time with 12 per cent. hydrochloric acid solution and then tested with aniline acetate. No furfural reaction was obtained. The extract does not appear, then, to contain substances of a pentose or pentosan nature which might give rise to furfural or its derivatives on hydrolysis.



For purpose of comparison a similar distillation was made with 25 grams of peat, this time under ordinary pressure, and the same tests were carried out on the aqueous extract of the tar. No furfural could be detected, nor could any solid benzoyl derivative be obtained, and no reduction of Fehling's solution occurred either before or after hydrolysis. On the other hand, reductions of alkaline permanganate and ammoniacal silver nitrate were obtained. It is possible, however, that these two are due to traces of formaldehyde and other aldehydes formed in both distillations.

Summary.

The method of rapid vacuum distillation has been applied to the investigation of dry peat. A comparison of the products formed with those which arise in the ordinary pressure distillation of peat, shows a decrease in the amounts of ammonia, acetic acid, methyl alcohol, and gases, and an increase in the tar, particularly in the higher boiling or more paraffinic fraction. This points to a milder type of decomposition under reduced pressure, in which the more complex intermediate products are less drastically broken down, and the formation of the simpler and more volatile end-products is lessened. It is probable that the cellulose content of the peat is responsible for the differences in the reactions obtained in the two methods of distillation, noted in the last part of the paper, but on account of the complexity and non-homogeneity of peat, it might be well to carry out work separately on the humic, lignocellulose, and other fractions which compose it before arriving at any more definite conclusions. This side of the question is receiving attention at present in these laboratories, and initial experiments with humic acid and lignocellulose, both from peat, confirm these views. Detailed results will be reported on later.

No. 28.

VELOCITY OF FORMATION OF 3·5-DIMETHYLPYRAZOLE-4-DIAZONIUM CHLORIDE.

By JOSEPH REILLY, M.A., D.Sc., F.R.C.Sc.I., F.I.C.,

AND

HONORAH E. BASTIBLE, B.Sc.

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While the nature of the diazo-reaction with regard to aromatic compounds has been extensively studied, both from the chemical and physico-chemical standpoints, its significance with regard to heterocyclic amines has received less attention. In connection with the constitution of diazonium salts, it is interesting to notice that certain heterocyclic amino-compounds on diazotisation behave very similarly to aniline as far as their chemical reactions are concerned, though this is not a general rule.

It has been shown recently (Reilly and Madden, Trans. Chem. Soc., 1925, **127**, p. 2936) that heterocyclic diazonium salts from 4-amino-3·5-dimethylpyrazole (e.g. 3·5-dimethylpyrazole-4-diazonium chloride and nitrate) are considerably more stable than any aromatic diazonium salts hitherto prepared. No work, however, appears to have been carried out on the rate at which these, or other heterocyclic diazonium salts, are formed; and in this paper the authors describe experiments of this nature. The rate of formation of diazonium salts was ascertained by estimating the amounts of nitrous acid present at varying intervals in a reacting mixture of definite amounts of base and sodium nitrite and mineral acid. The quantity of nitrous acid found was a measure of the amounts of the diazonium salts which were formed in certain time intervals, and so was a measure of the rate of the reaction. To confirm that the nitrous acid had reacted to form a diazonium salt, and had not changed otherwise, check experiments were carried out, in which the azo compound with β-naphthol, formed from the diazonium salt prepared at different temperatures, was identified and weighed.

Owing to the very rapid formation of diazonium salts in concentrated solutions, very dilute solutions were employed, the reaction being carried out in N/1,000 solutions, and the estimations made in N/100,000 solutions. The effect of this dilution is to retard greatly the rate of the reaction, and so render it capable of more accurate measurement. The method of procedure closely followed that employed by Hantzch and Schumann for aniline (Ber., 1899, **32**, p. 1691), except that the colorimetric estimation of nitrous acid, instead of being made in ordinary standard Nessler tubes, was carried out in a modified Klett Bio-colorimeter of the Duboscq type. Potassium iodide was used in preference to zinc iodide, as this latter reagent is extremely sensitive, and is influenced by the presence of even traces of impurities. In the Klett instrument the solutions are placed in cups, the bottoms of which are fused, and are free from optical

defects. Dipping into these cups are fixed plungers, which, like the cups, have opaque walls and transparent bottoms. The cups are carried on stages which are movable. Two millimetre scales, with verniers attached, are used to measure the heights of the liquid columns, which decrease as the cups are moved upwards. When the cups are screwed up so that they make contact with the bottom of the plunger, the verniers should both read zero. These verniers are movable, and so are capable of adjustment.

Diffused daylight is reflected up by mirrors through the two solutions to the top of the plungers. These reflectors work independently of each other, and the reflected light can be adjusted so as to ensure a very even field. The rays from the two sides are brought to a common field by means of an observing microscope, which also allows the fine dividing line to be sharply focussed. The depth of the standard solution is set at a convenient mark, the depth of the other being varied by means of the coarse and fine adjustments until the two halves of the field are identical in appearance. A reflecting mirror at the top allows the scales to be read from the same point of observation as the colour fields.

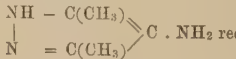
The necessary calculations are simple. The assumption is that the depth of colour is proportional to the concentration of the substance. The depths of the solutions, therefore, vary inversely as the concentrations in the standard and in the unknown respectively. If the standard contains a certain amount of material in a given volume, and the unknown is made up to the same volume, then from the readings of the standard and the unknown the amount in the latter can be calculated.

Preparation of 4-amino-3·5-dimethylpyrazole.

5 grams of 3·5-dimethylpyrazole were heated with 1·7 c.c. of sulphuric acid (d. 1·84) and 4·2 c.c. of fuming nitric acid (d. 1·5) until nitrous fumes were freely evolved. The reaction mixture was cooled, and after a short period again heated. The nitrated product was poured on to ice, and the solution nearly neutralised with sodium hydroxide, when 4-nitro-3·5-dimethylpyrazole separated. The nitro compound was reduced to 4-amino-3·5-dimethylpyrazole by means of an aluminium mercury couple in moist ether, as described by Morgan and Ackermann (Chem. Soc. Trans., 1923, **123**, p. 1311).

On recrystallising from ethyl alcohol, the base was obtained in the form of white crystals, which melted with decomposition about 202° C.

Found N = 37·8 per cent.



Diazotisation of 4-amino-3·5-dimethylpyrazole.

In the first instance N/10 solutions of 4-amino-3·5-dimethylpyrazole hydrochloride and of sodium nitrite were prepared. The sodium nitrite used was recrystallised "pure" Kahlbaum Sodium Nitrite. On a quantitative test it was shown to be over 99 per cent. pure. For each diazotisation 10 c.c. of the N/10 sodium nitrite was made up to N/500 sodium nitrite. Similarly, N/500 solution of hydrochloride of the base was made up, excess acid, as required for different estimations, being added during the dilution.

500 c.c. of each of these two solutions on mixing gave a litre of N/1,000 solution of the diazotisation mixture. In estimating the nitrous acid at varying

times, 1 to 2 c.c. of the reaction mixture was run into a 100 c.c. vessel containing 3 c.c. of the potassium iodide starch solution (the stock solution contained 4 grams of starch and 2 grams of potassium iodide per litre), 1 c.c. of dilute sulphuric acid was added, and the whole was made up to 100 c.c. This was compared with a standard solution, made up to 100 c.c. in a similar vessel, and containing, in addition to the potassium iodide, starch, and sulphuric acid, 1 c.c. of N/1,000 sodium nitrite solution. The nitrous acid was estimated in N/100,000 solution. The two vessels were allowed to stand, since the blue colour is not formed at once in these very dilute solutions. In about 15 minutes the colour becomes very intense, and is then most suitable for comparison. After several hours the colour varies, so it is advisable to carry out the estimations within an hour, at most, of mixing the solutions.

The solutions were compared by means of the Klett colorimeter. The standard solution was set at 20 mm. If to give the same intensity of colour on both sides of the divided field the unknown solution required a depth of 30 mm., then its strength was two-thirds that of the standard. The percentage of nitrous acid present, then, could always be found by comparison with a known standard. As the reaction proceeds and the blue colour becomes more faint, the standard, in each case, can be diluted to half or quarter strength, thus rendering it, to some extent, comparable with the intensity of the solution to be estimated. The colorimeter can compare to one-third of the strength of the standard solution, but it gives more accurate results with solutions of about the same strength as the standard.

The formation of the diazonium salt proceeds somewhat irregularly during the first few minutes, so the speed of the reaction can only be calculated with a fair degree of accuracy after a certain interval.

At the same time as the experiments were carried out on the pyrazole base a control was done with aniline. The experiment was carried out first with no excess, i.e. with one molecule of the hydrochloride of the base to one molecule of nitrous acid, and gave the following results:—

Experiment with no excess of acid.
Temp. 0° C.

	Aniline.		4-amino-3·5-dimethylpyrazole.	
Time in mins.	Nitrous Acid reacted.	Velocity Const. K.	Nitrous Acid reacted.	Velocity Const. K.
	p.c.		p.c.	
30	45	0·027	48	0·030
60	60	0·025	56	0·021
120	73	0·023	73	0·023
180	80	0·022	80	0·022
240	—	—	86	0·026
	Average value of K = 0·024.		Average value of K = 0·024.	

The reaction appears to be bimolecular. K was calculated from the well-known formula

$$K = \frac{x}{a(a-x)} \times \frac{1}{t}.$$

Experiments with excess of acid.

The solution of the base was again made up in each case, this time with one molecule of acid in excess, i.e. one molecule of the hydrochloride of the base to one molecule of nitrous acid, as well as one free molecule of hydrochloric acid. The experiments with aniline and 4-amino-3·5-dimethylpyrazole were again carried out at 0° C. under these conditions.

Temp. 0° C.

	Aniline.		4-amino-3·5-dimethylpyrazole.	
Time in mins.	Nitrous Acid reacted.	Velocity Const. K.	Nitrous Acid reacted.	Velocity Const. K.
	p.c.		p.c.	
30	53	0·037	60	0·05
60	69	0·037	65	0·031
120	80	0·033	78·7	0·031
180	86	0·034	85	0·031
240	—	—	88	0·030
	Average value of K = 0·035.		Average value of K (omitting first value) = 0·031.	

The speed of the reaction with 4-amino-3·5-dimethylpyrazole is thus seen to approximate closely to the rate of diazotisation of aniline. The addition of excess hydrochloric acid (1 mol.) increases the speed of the reaction in each case.

The Effect of Temperature on the Velocity of Reaction.

The velocity of reaction was also measured at 11° C., 21·2° C., 34° C., 50° C., 58·5° C., and 72° C. These records are of interest, for in no other case, even among aromatic amines, has such an extended series of results been obtained. In fact, the only recorded result which we have seen has been one for bromaniline, in which the rate of reaction in diazotisation has been determined at 10° C. and 20° C. The rate of the reaction between nitrous acid and 4-amino-3·5-dimethylpyrazole hydrochloride increased with rise of temperature, as shown below.

The following results have been obtained at 11° C.:—T = 30, y = 64 (K = 0·059). T = 60, y = 77 (K = 0·055). T = 120, y = 87·5 (K = 0·058). T = 180, y = 90 (K = 0·050). T = 240, y = 92·3 (K = 0·050). Where T = time in minutes, y = per cent. nitrous acid reacted, and K = velocity constant. Average value of K = 0·054.

The reaction was next carried out at 21·2° C., the temperature being kept constant by immersing the beaker in a thermostat:—T = 10, y = 58 (K = 0·138). T = 15, y = 66·7 (K = 0·134). T = 20, y = 72·8 (K = 0·134). T = 30, y = 80·4 (K = 0·137). T = 40, y = 84·8 (K = 0·139). T = 45, y = 85·3 (K = 0·129). T = 50, y = 87 (K = 0·134). T = 60, y = 87·5 (K = 0·120). T = 70, y = 91 (K = 0·144). T = 70, y = 91 (K = 0·144). T = 120, y = 94·2 (K = 0·130). T = 180, y = 96 (K = 0·133). Average value of K = 0·135.

As the reaction was going more rapidly, after the first control experiment, readings were taken at shorter time intervals. The temperature of the thermostat was now raised to 35° C., which gave a constant temperature of 34° C. in the diazotisation mixture. The reaction was

carried out as before, readings being taken at the time intervals indicated:— $T = 5$, $y = 63·4$ ($K = 0·35$). $T = 10$, $y = 80·8$ ($K = 0·42$). $T = 15$, $y = 86$ ($K = 0·41$). $T = 20$, $y = 90·5$ ($K = 0·48$). $T = 25$, $y = 91·7$ ($K = 0·44$). $T = 30$, $y = 92$ ($K = 0·43$). $T = 60$, $y = 96$ ($K = 0·41$). $T = 130$, $y = 98·1$ ($K = 0·40$). Average value of $K = 0·42$.

The next determination was taken at 50° C., the thermostat which contained the vessels with the nitrite and base registering 51·5° C. A control experiment to determine the stability of an N/1,000 solution of nitrous acid alone was first performed at this temperature. The solution was heated for an hour, and tested at varying time intervals. Under these conditions the nitrous acid solution showed little change, and the slight alterations would have no appreciable effect on the results for K at 50° C.

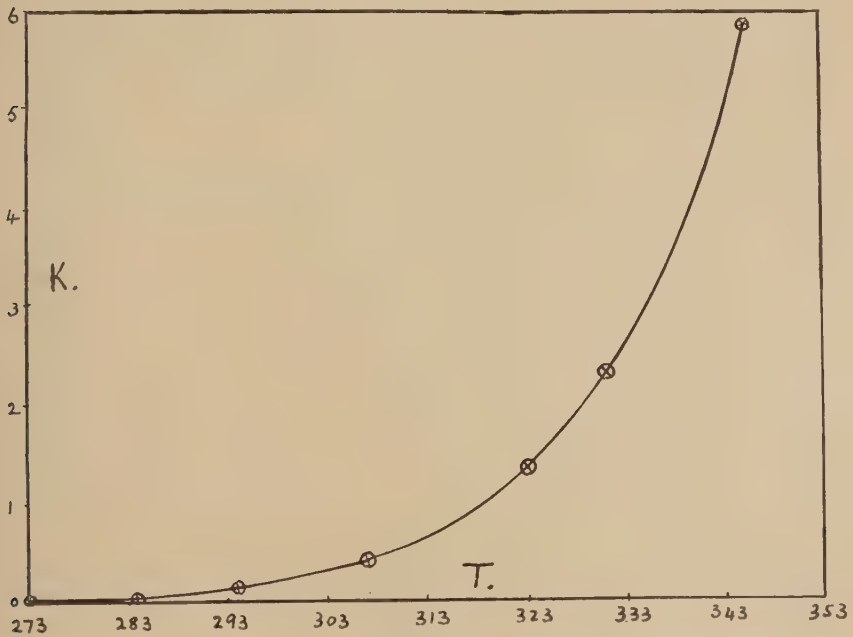


Fig. 1.

Estimations of the amount of nitrous acid present in the "diazo mixture" were carried out at shorter time intervals, owing to the increased speed of the reaction, 97 per cent. of the acid having reacted after 25 minutes at this temperature:— $T = 2$, $y = 73·5$ ($K = 1·38$). $T = 5$, $y = 87·3$ ($K = 1·37$). $T = 10$, $y = 93$ ($K = 1·33$). $T = 15$, $y = 95·3$, ($K = 1·35$). $T = 20$, $y = 96·4$ ($K = 1·34$). $T = 25$, $y = 97·2$ ($K = 1·38$). Average value of $K = 1·36$.

The temperature of the thermostat was now raised to 60° C., the temperature of the solution being constant at 58·5° C. A control experiment on the nitrous acid was again done at this temperature. The decrease in nitrous acid was small after a half hour, and only a very slight difference in the value of K would result from this loss:—$T = 2$, $y = 81$ ($K = 2·17$). $T = 6$, $y = 93$ ($K = 2·21$).

$T = 10$, $y = 96$ ($K = 2{\cdot}40$). $T = 15$, $y = 97{\cdot}3$ ($K = 2{\cdot}34$). $T = 20$, $y = 98$ ($K = 2{\cdot}45$). Average value of $K = 2{\cdot}31$.

A control experiment of the following nature was carried out at this stage. 0·5 gram of 4-amino-3·5-dimethylpyrazole was diazotised in the ordinary way at 0° C. with sodium nitrite (slight excess of 1 mol.) and hydrochloric acid (3 mols.). The excess of nitrous acid was removed by carbamide, and the diazonium chloride added to an alkaline solution of β-naphthol. The resulting azo-β-naphthol compound was washed, dried, and weighed (1·193 grams). A second experiment was carried out, with one alteration, namely, that the diazotisation was carried out at 50° C. The resulting product with β-naphthol was only slightly less in weight, 1·11 grams, than that obtained at 0° C., and on recrystallisation from ethyl alcohol the resulting products were similar, and agreed with the 3·5-dimethylpyrazole-4-azo-β-naphthol (M.P. 251°) prepared by Reilly and Morgan (*ibid.*). This experiment is of importance, as it proved that in the reaction at 50° C. the nitrous acid brings about diazotisation normally, and is not otherwise used up.

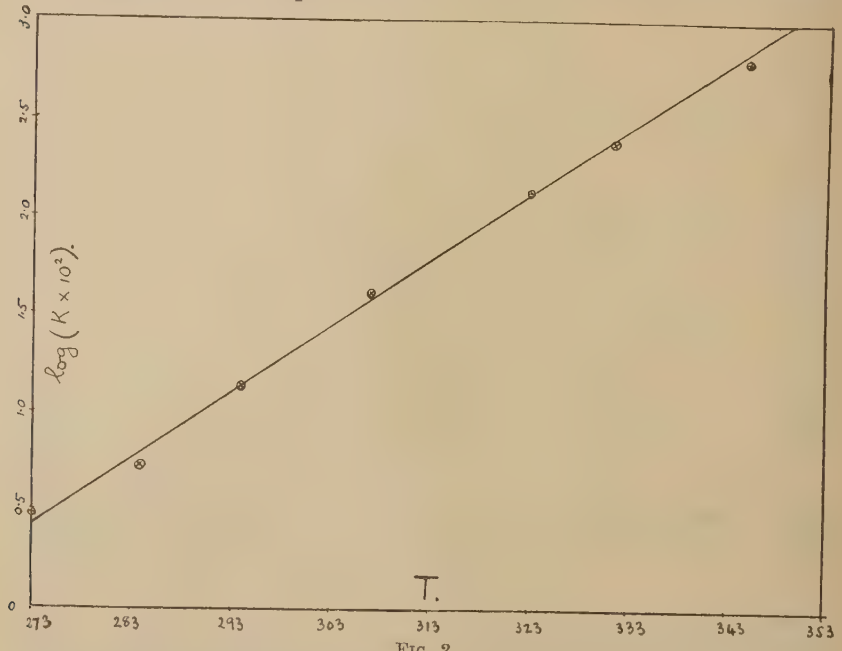


FIG. 2.

The final determination was carried out at 72° C. The reaction was very rapid in this case, approximately 92 per cent. of the nitrous acid having reacted in a period of two minutes ($K = 5{\cdot}8$). In a control experiment for this period in the absence of the base, the nitrous acid had not altered sufficiently to influence the value of K appreciably.

As shown in fig. 1, with increase of temperature there is a marked rise in the value of K. On plotting log ($K \times 10^2$) against T, absolute, the points are found to lie approximately on a straight line, as shown in fig. 2.

The equation of this line is

$$\log_{10}(K \times 10^2) = \frac{1}{30}\,T' + 0{\cdot}433,$$

where T' = actual temperature, Centigrade, at which the reactions were measured,

$$\text{i.e.}\quad \log_{10}(K \times 10^2) = \frac{1}{30}\,T' + \log 2{\cdot}71\,;$$

$$\therefore\quad K = 0{\cdot}0271 \times 10^{\frac{T'}{30}},$$

$$\text{or}\quad K = 0{\cdot}0271 \times e^{\frac{T'}{13}}.$$

It is intended to extend this work so as to include other amino-heterocyclic compounds.

Chemical Department,
University College, Cork.

No. 29.

RESPONSES OF PLANT TISSUES TO ELECTRIC CURRENTS.

By H. H. DIXON, Sc.D., F.R.S.,
Professor of Botany in the University of Dublin,

AND

T. A. BENNET-CLARK, B.A.,
Assistant to the Professor of Botany in the University of Dublin.

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Introduction.

The change of resistance of a tissue consequent on the passage of an electric current through it seems to offer a suitable method for the quantitative investigation of the relation between stimulus and response. It has promise of shedding light, also, on the nature of the protoplasmic membrane, and on the mechanism of the control of permeability. In so far as a change in resistance depends on a change in permeability, this method offers a means of conveniently measuring what has been regarded as the most fundamental response.

Stiles' (8) objection, that alteration in the conductivity of the tissue bears little relation to alteration in the permeability, since changes in the concentration of the electrolytes will alter the conductivity without affecting the permeability, can scarcely affect the results to be described in this paper, as it would involve the assumption of very rapid changes in concentration. For these changes there is no evidence. It must be pointed out also that these objections to the electrical conductivity method of measuring permeability apply with equal force to all other methods, as these depend on either the osmotic pressure of the cell-sap or on the gradient of concentration of some substance across the cell-membranes.

Experimental Methods.

Various modifications of the Kohlrausch Bridge have been used in the investigation of the electrical resistance of tissues; all of them apparently give satisfactory results, except where the cross-section of the tissue is so restricted that the resistance is very high: then a long silent region, or one where the sound is uniform, develops on the ratio wire. This renders the true balancing-point difficult to ascertain: it is found that this region is more extended if the leads introducing the tissue into the bridge are twisted together than if they have independent courses.

It is necessary that the buzzer should deliver a very small current which will not itself stimulate the tissues. In our experiments the current was so small that it could be scarcely detected when the electrodes were placed upon the tongue. Furthermore, when this current was passed through an experimental piece of tissue for one hour, no alteration of resistance could be detected.

It has been observed already (1) that temperature has a large effect on the

resistance of tissues, hence it is of importance to carry out each series of experiments at a constant temperature. Few, if any, previous investigators realised the necessity for this precaution.

Previous investigators used either induction shocks or prolonged currents. With induction shocks both the voltage and the quantity of energy supplied are unknown, and are, moreover, subject to variations due to the variable rapidity of making and breaking contact.

For any considerable duration of stimulation, alternating currents are essential, so that polarisation effects may be kept as low as possible.

We have used the alternating current from the city supply, which has a frequency of 50 cycles per second. The virtual voltage is 200, and, as the change of voltage approximately follows a sine curve, the maximum voltage is $\sqrt{2}$ times the virtual voltage. The voltages recorded in this paper are virtual voltages, as read on a voltmeter. In order to obtain voltages between 0 and 200, this alternating current is led through a potentiometer (C, fig. 1) consisting of four "Zenith" rheostats (W), each having a resistance of about 33 ohms. A slider (P) may make contact with any point of the potentiometer and lead off a current of the desired voltage. The circuit is completed through the tissue, and a voltmeter (B) connected in parallel shows the effective voltage used.

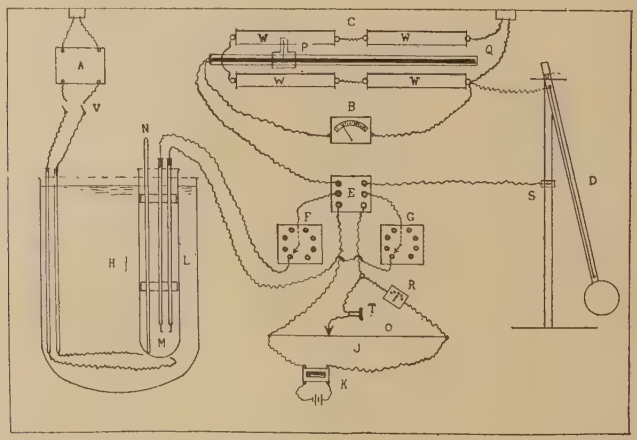

Fig. 1.

Into this circuit is also introduced a device for controlling the duration of stimulation. This device consists essentially of a pendulum (D) about a metre in length with a heavy leaden bob weighing about 10 lbs. Along this pendulum a copper wire is stretched, and, as the pendulum swings, this rubs past a short copper contact; the contact (S), which is held against the wire by a spring, is adjustable in height, so that it may make connection with any point of the wire on the pendulum: of course, the nearer the point of contact is to the pivot of the pendulum the longer will be the duration of the current. A simple latch release starts the pendulum at a fixed divergence from the vertical. The exact duration of the current is determined by the vibrations of a tuning-fork recorded on a smoked cover-glass fixed at the same level on the pendulum as the contact.

In the circuit there is also a control switch (E) which remains open until the pendulum is latched back into its starting position: the switch is then closed, and, as soon as the wire on the pendulum has rubbed past the contact, the control switch is opened. By this means the passage of the current may be conveniently limited from 1/5 to 1/50 of a second: when currents of longer duration are required, a light arm on the bob of the pendulum is arranged to tip over a balanced contact, and complete the circuit, on its outward journey, and to tip this contact back on its return, thus breaking the circuit.

The tissues, whose change in resistance is to be measured, are supported on platinum electrodes which have been coated with platinum black to diminish polarisation effects. Each of these platinum electrodes is formed out of a half-centimetre square of platinum foil. At right angles to one side of this a piece of platinum wire is attached, and this is fused into the end of a quill glass tube which is then filled with mercury. Two pairs of these electrodes are rigidly supported in the lower end of a large test-tube (L) 4 cm. in diameter and 30 cm. long. They are held in position by passing their glass tubes through two or three discs of cork, about 4 or 8 cm. apart, which fit the test-tube closely. In addition to the four glass tubes of the electrodes, a glass rod (N) is passed through the cork discs, and this, resting on the bottom of the test-tube, prevents the electrodes from coming in contact with the latter. The glass rod projects above the test-tube, and furnishes a handle by which the framework of electrodes and cork discs can be removed from the tube.

It was usually arranged that the two electrodes of each pair were 1 cm. apart, and the platinum foil of each was bent so as to form a horizontal ledge on which the piece of tissue would rest. A uniform coating of platinum black was then deposited on the electrodes, which, after washing, were ready for use. The pieces of tissue, which were cut one centimetre square, were laid on the ledges on the electrodes; these had been previously charged with Knop's solution to make good contact between the electrodes and the tissue. To prevent spreading of the Knop's solution the upper and lower surfaces of the tissue were smeared with vaseline before cutting: this was effective only when leaves were used.

In order that the tissues may be kept at a suitable constant temperature during the experiment, the test tube is supported in a glass vessel (H) containing several litres of water. This water is maintained at the required temperature by an adjustable current passing through an annular wire resistance placed in the bottom of the glass vessel near its wall. This secures that any desired temperature may be maintained with fluctuations of less than 1° C. for several hours. Several test tubes, each with two pairs of electrodes, may be placed in this thermostat.

In our apparatus the heating current was supplied by transforming the city alternating current down to about 25 volts; this current was further regulated by a rheostat. It was found that when the current in the primary of the transformer was broken, the tissues in the thermostat behaved as though a current had passed through them: it is supposed that a high E.M.F. was induced in the secondary of the transformer, and that the current from it leaked into the tissue. This involuntary shocking of the tissues was prevented by disconnecting both poles of the secondary of the transformer from the thermostat by a switch (V) when any change had to be made in the current flowing in the primary.

Several squares of tissue, set up as described, were placed in the thermostat together. Suitable leads with their terminals dipping into the mercury filling the tubes of the electrodes were connected with the binding screws of two dial

switches (F and G), so that each square of tissue might be in turn introduced into one arm of the Kohlrausch Bridge (J) while the other is occupied by an adjustable resistance of 0 – 200,000 ohms (R).

Experimental Results.

The chief desiderata in the material to be investigated are (*a*) that the tissue should be homogeneous, (*b*) that considerable quantities of pieces similar in resistance and sensitivity should be readily obtainable, and (*c*) that the saline solution used to make contact should not creep over the surface making a short-circuit.

This last condition was found to exclude all pieces of tissue cut out of solid organs, such as the fruit of the apple, potato tubers, and also the thalli of liveworts and algae: in any of these fairly uniform tissue may be obtained, but the point of balance on the Kohlrausch Bridge was found occasionally to move very suddenly as much as 10 cm., indicating extremely rapid changes of resistance: these were supposed to be due to the formation and breaking of the films of Knop's solution which probably covered the surface of the tissues above mentioned.

The only really satisfactory materials we have found are leaves, and the following observations have been made on the leaves of an ivy (*Hedera helix*) plant growing near the School of Botany: a few have been made on the leaves of *Sedum praealtum*.

The ivy possesses an advantage over the Sedum in that it remains in good condition in the apparatus for as long as two days, on the other hand, however, its tissue is not so homogeneous.

Pieces of leaves 1 cm. square, vaselined on the surface, were placed in the apparatus at a temperature of 29–31° C. except where otherwise stated.

On setting up, the resistance is found to fall off rapidly at first, then more slowly, till after about twenty minutes it becomes constant, fluctuating seldom more than 2 per cent. above and below a level value. This falling of resistance is probably due to the stimulus of cutting, the contact of the saline solution, and the rise in temperature of the leaf. Some of the squares set up never acquired a constant resistance, but continued to fall slowly, this fall being often associated with the gradual injection of the intercellular spaces: all such squares were rejected.

In a previous communication one of us (1) showed that the resistance of ivy leaf squares varied considerably with temperature, and also with the age of the leaf and with the season of the year, these results have been confirmed, and the variations in sensitivity with variations in these external conditions will be considered later.

The range of variation in resistance of ivy leaf squares is shown graphically in the frequency-distribution polygons given in fig. 2. These it will be seen relate to somewhat small numbers of individuals, 64, 27, and 40 respectively. The observations compiled in each were made at 29–30° C. and at the following dates:—March 19–27 (continuous curve), February 6–February 14 (broken curve), and December 9–19 (chain curve).

These polygons are all somewhat asymmetrical, the asymmetry being due to a number of leaf squares of particularly low resistance. The resistances of the "abnormal" leaves are lower in the series examined in March than in the other two, and they differ more markedly from the normal leaves then than in December and February.

Two classes of leaves, responding to stimuli to markedly different extents, are distinguishable in the later seasons, but this is not the case earlier: squares

of low resistance, which cause the secondary maxima in the polygons of fig. 2, give different responses from the "normal" leaf squares (see the broken curve of fig. 4, and the description on p. 356).

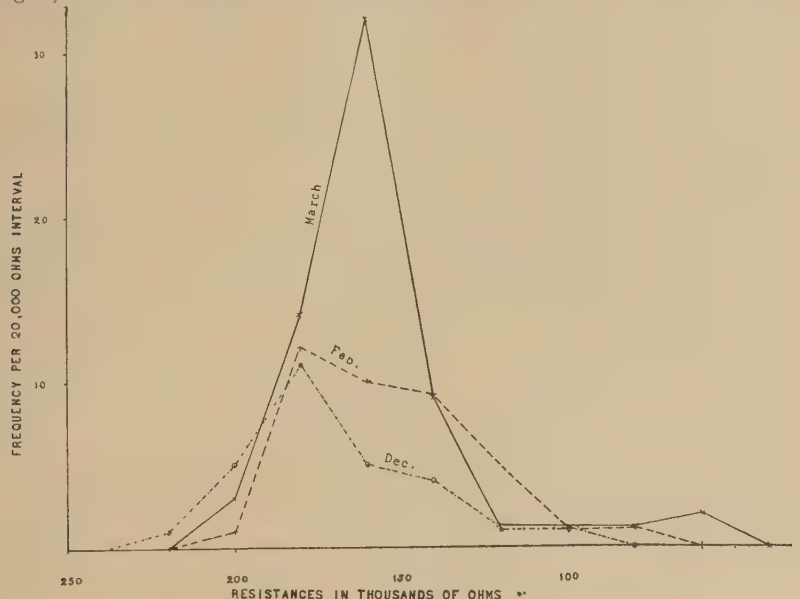

FIG. 2.

Nature of the Response.

The effect on the resistance of a normal leaf of the passage of a current through it, is indicated graphically in fig. 3. The times are plotted as abscissae

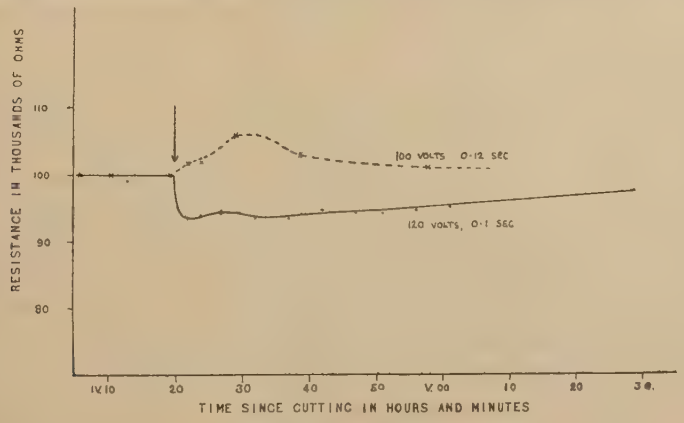

FIG. 3.

in hours and minutes, the time of cutting being reckoned as zero hours; the resistances, in thousands of ohms, are plotted as ordinates.

In the case of the record shown as the continuous curve in fig. 3, the value of the resistance prior to stimulation was 166,700 ohms; but, in order to facilitate comparison of this experiment with other experiments, this level value is reduced to 100,000 ohms, all the other resistances being divided, correspondingly, by 1·667. The continuous curve is the record of the "reduced" resistances of a square of leaf on March 22nd.

The resistance was read at intervals from the time of cutting, taken as 0 hours, for about 6 hours. A stimulus of 120 volts, lasting 0·1 second, was given at the time indicated by the arrow. It has been pointed out that since an alternating current was used, the maximum voltage will be $\sqrt{2}$ times the virtual voltage.

Immediately after the stimulus the resistance is found to fall rapidly at first, then more and more slowly, till after about 5 minutes there is no further fall. Almost always, but especially when the response is small, a slight rise and then another fall follow the initial fall in resistance; after remaining for some time at a low value the resistance begins to rise gradually, attaining, after about an hour or more, the same value as that obtaining before stimulation.

A change is thus seen to be initiated by the stimulus, which develops with gradually decreasing rapidity to its maximum value; this is then followed by a very slow return to the original state. Superposed on this reaction is another one in the opposite sense. These other reactions, which we will call positive reactions, are less noticeable the larger the stimulus, and *vice versa*.

The maximum deviation from the original resistance is attained at from 2 to 20 minutes after stimulation, the time being longer the greater the deviation; this maximum deviation will be called, for convenience of description, the response: and throughout this paper it is expressed as a percentage of the original resistance. In the example above the response is – 6 per cent. The largest possible response is about – 96 per cent., since the dead resistance of one of the ivy-leaf squares is about 5,000–7,000 ohms.

The variations in the resistances of the 1 cm. squares of leaf, as shown in fig. 2, are due to two factors:—variation in the area of cross-section of the squares of leaf and variation in the absolute conductivity of the component cells. Variations caused by the former will not affect the current density in the leaf when the current is supplied at a given voltage, whereas in a leaf having a greater conductivity there will be a greater current density than in a leaf of smaller conductivity.

A typical frequency-distribution polygon of the responses to a stimulus of 120 volts, lasting 0·1 second, on March 22nd, is shown by unbroken lines in fig. 4. All these results refer to leaves of "normal" resistance, as described before.

The "abnormal" leaf-squares, which have particularly low resistances, pass much larger currents than the "normal" ones for the same voltages. Notwithstanding the fact that the currents stimulating them vary from 0·8 to 2·4 milliamperes, all their responses fall quite close to – 15 per cent., as seen in the dotted frequency-distribution polygon in fig. 4. These leaves do not show nearly such large responses as normal leaves through which the same quantity of current is passed, when this current is obtained by an increased voltage acting for the same time: the responses are slightly smaller than those of normal leaves through which the same quantity of current is passed by an increase in the duration of the stimulus. The term, "quantity of current," is used here to denote the product of the current and the duration. Since alternating currents are used, the quantity of electricity passed during stimulation will be nil, if the current is in the same phase at the beginning and at the end of the stimulus.

Certain differences in the characters of the reactions obtained with stimuli of different magnitudes must be now considered.

When the responses have been greater than – 40 per cent., we have failed to observe complete recovery; this is probably due to some lethal change setting in before the original state could be attained. The larger the response the longer was the time taken by the leaf-square to recover.

When the responses have been greater than – 70 per cent., no recovery could be detected, and, in addition, the leaf-square was partly blackened, the discoloration being darker the greater the fall in resistance.

With stimuli smaller than a certain value at which no response is obtained, the positive reactions, already referred to, become more marked than the negative reactions, and the resulting response is positive instead of negative. Such a response is shown graphically in the broken curve of fig. 3; the stimulus in this case was 100 volts, lasting 0·12 second.

The effect of a negative reaction, which reaches its maximum earlier than the maximum of the positive reaction, is seen as a depression in the rising part of the curve.

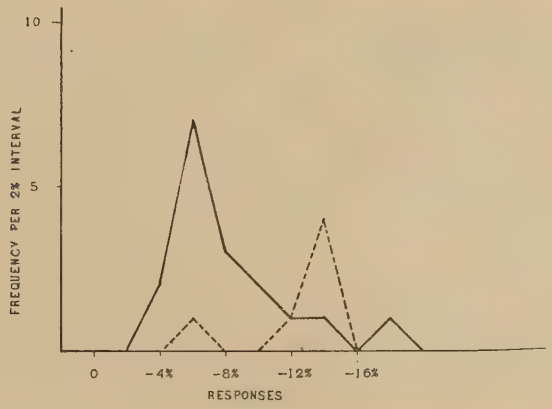

Fig. 4.

Quantitative Relations between the Responses and the Voltage and Duration of Stimulation.

Throughout this description the following units will be used:—Responses (taken as the greatest deviations of the resistance from the normal) are expressed as percentages of the normal resistances; the current is expressed as milliamperes per cross-section of tissue, of which the resistance is 100,000 ohms when its length is 1 cm. This current has been calculated from the observed values of the voltage and resistance. The very small inductance and capacity have been neglected. In fig. 5 the relations between the responses and the voltages of the stimuli, where the stimuli lasted for 4 secs., 0·8 sec., and 0·1 sec., respectively, are shown as unbroken curves; the results embodied in these curves were obtained during the last ten days of March. The dotted curves represent the results of experiments made at the end of February; stimuli of 0·1 and 0·02 sec. were used at the earlier date, and the voltages applied are shown as abscissae, the corresponding responses being shown as ordinates.

Each curve represents the results of about three dozen observations, except that for 0·1 sec. in March, in which each point is the mean of eight observations on

"normal" leaves. The points on the February curves have been obtained from smoothed "response-duration" curves, which will be described later.

In obtaining these data, no leaf received more than one stimulus; this was considered advisable, lest some disturbance should remain which was not detectible as an alteration in the resistance.

Since the potential of the stimulating current fluctuates in a sinusoidal manner, its effects might conceivably differ according to the phase of the current at the commencement and end of the stimulus. If the stimulus starts when the potential is zero, one would expect a smaller response than if it were to start when the potential was at its maximum, as, in the latter case, the rate of change of current will be greater at the beginning of the stimulus. This rate of rise of the current will be very rapid, since the inductance and capacity of the tissue are negligibly small. The effect will, however, be masked to a great extent by the rapid rates of change of current during the stimulus, except, perhaps, when the stimuli are very small.

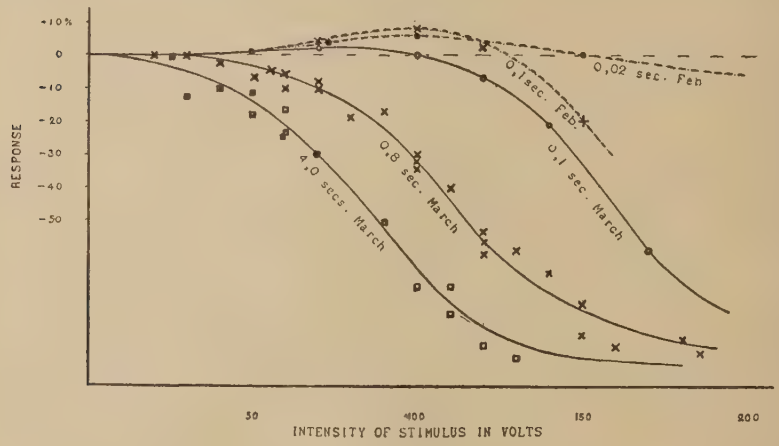

Fig. 5.

According to Keith Lucas (5), in nerve and muscle the effect of an alteration in the current-gradient on the efficacy of a stimulus of given magnitude, becomes less and less noticeable the greater the current-gradient; in other words, an increase in the rate of change of the current (current-gradient), when this is low, will have a considerable effect on the efficacy of the stimulus, but a corresponding increase when the gradient is high (as it is in our work) will have no effect on its efficacy.

We hope later to extend this work to an investigation of the influence of the current-gradient on the effectiveness of stimuli of definite energy contents.

The irregularity in the responses to small stimuli observed by us is, in part, probably connected with the uncertainty as to the phase of the current at the commencement of the stimulus. Also, as has already been noted, if the phase at which it ended was not the same as that at which it began, a small quantity of electricity must have passed from pole to pole.

Referring again to fig. 5 and the continuous curve marked 0·8 sec., it will be seen that the responses are but little affected by given increments in the voltage when this is low, but that at higher voltages (40 volts and over) larger and larger

effects are produced in the response by given increments in the stimulus, until this rises to 110 volts; from this point onwards equal increments in the stimulus produce successively smaller and smaller increments in the response: stimuli of 0·8 sec. and 200 volts produce nearly as big a drop in resistance as can be produced.

It is also seen in fig. 5 that stimuli lasting 0·1 sec., of which the voltage is between 30 and 100, show a marked positive response, which is at its maximum about 70 volts; stimuli above 100 volts cause negative responses, which increase more and more rapidly up to the point of inflexion at about 155 volts; above that the behaviour is similar to that described for the longer stimuli. In the case of the dotted curve, labelled February, 0·1 sec., the experiments, although of the same duration, were conducted about a month earlier; here stimuli up to 120 volts produce positive responses, the largest (+ 8 per cent.) being at 100 volts.

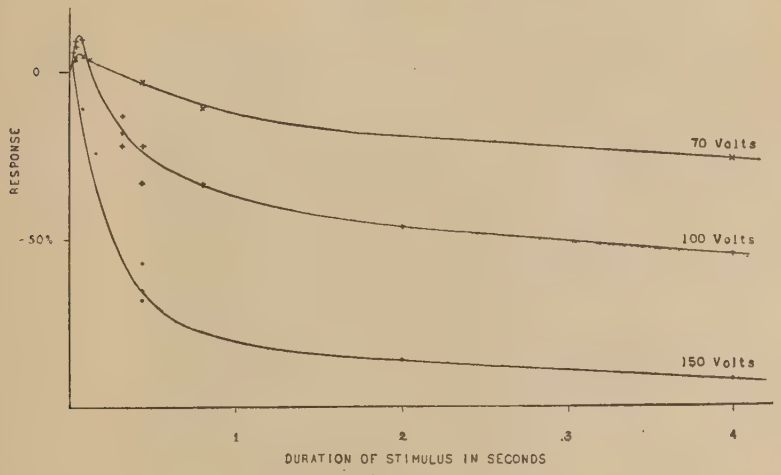

FIG. 6.

The influence of the season is very marked. This increase in sensitivity of the tissue as summer approaches will be referred to again; at present it will suffice to say that 0·1 sec. in February must be regarded as a smaller stimulus than the same duration of stimulus in March.

The magnitudes of the responses to still smaller stimuli of different voltages, lasting for 0·02 sec. in February, are shown in the dotted curve.

In a series of experiments made in February, definite voltages were applied for various times. In fig. 6 three curves are shown, where 150, 100, and 70 volts were used, and the times for which they acted are plotted as abscissae; the corresponding responses are plotted as ordinates.

When 150 volts were applied for 0·02 sec., it was found that no response was obtained, but 150 volts for 0·04 sec. caused a fall in resistance of about 6 per cent.; further equal increments in the durations caused successively smaller increments in the responses. With a lower voltage (100 volts), up to a duration of 0·14 sec., the response is positive, the maximum (+ 10 per cent.) being obtained with a duration of 0·08 sec. Longer stimuli than 0·14 sec. bring about negative responses, equal increases in the durations causing smaller and smaller increases in the responses. Stimuli of 70 volts for different times show essentially similar

features; but it is to be noted that the maximum increase in resistance is smaller than with 100 volts, and, with increasing durations, the responses fall to zero and below zero much more slowly than was the case with higher voltages.

These results are expressed in a third way in fig. 7; here the voltage is measured along the vertical axis, and the time for which it must act to produce responses of – 80 per cent., – 50 per cent., – 20 per cent., 0 per cent., + 1 per cent., + 4 per cent., and + 10 per cent. are plotted along the horizontal axis.

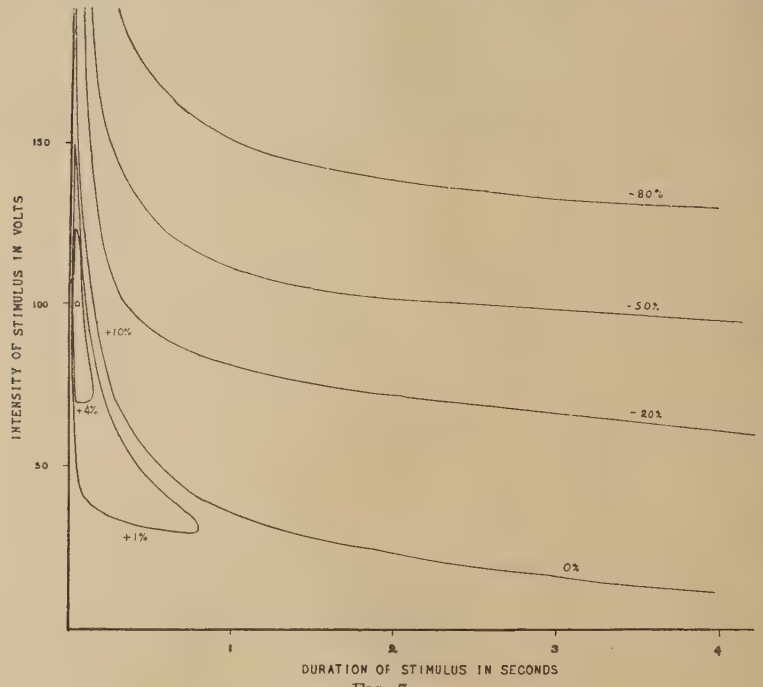

Fig. 7.

It is to be observed that the curves become steeper as the responses become smaller. The curve showing the time for which a given voltage must act to produce just no response (the "threshold" values of the stimulus) is marked 0 per cent. in fig. 7. This is not a true threshold value; these stimuli, which produce no change in resistance, do so merely because the positive and negative reactions are of equal magnitude.

To smaller stimuli than these the responses are positive. Curves are given showing what voltages and durations are required to produce responses of + 1 per cent., + 4 per cent., and + 10 per cent.; these curves are invariably closed, since at a given voltage there are two durations of stimulus at which the same response is obtained, and at a given duration two voltages which cause the same response. In fig. 7 above + 10 per cent. refers to the small closed curve on the ordinate, 100 volts.

It is hard to avoid the conclusion that the "threshold" curve becomes a closed curve by joining the two axes at infinity.

The data described in these three sets of curves can be more completely represented in the solid graph, of which two photographs are shown in fig. 8.

The origin is marked O, and the responses are measured on the vertical axis, points below O representing negative, those above, positive responses; the durations of stimulation are plotted along the axis OA, the length OA representing 5 seconds; the intensity of the stimulating current is plotted along OB, the length OB representing 200 volts.

The points in this surface, therefore, show the relations between the intensity and duration of the stimulus and the magnitude of the alteration in resistance that it causes.

The curves of fig. 5 are a series of sections through the surface parallel to the plane OBCD. Those of fig. 6 are sections parallel to AOD, and those of fig. 7 are horizontal sections; the position of the zero response contour is seen as a black line on the surface; this line presumably meets the axes OB and OA at infinity.

FIG. 8.

It will appear from the discussion at the end of the paper that this statement is strictly true only when we are dealing with tissues containing an infinite number of membranes, and does not apply when considering the responses of a single membrane.

At this stage it would hardly be of value to enter into much theoretical discussion of these curves; but their bearing on the Nernst-A. V. Hill (3, 6) theory of excitation should be mentioned. These workers suppose "excitation" to be brought about by a certain increase in the concentration of some ion or ions at some point near the semipermeable membranes of the excitable structure.

These membranes, in order to make the mathematical treatment of the problem possible, are assumed to be completely impermeable to electrolytes.

Making this assumption, which adds an error that cannot be determined, A. V. Hill and Keith Lucas (3, 4) show that the relations between the intensities and durations of the currents (direct currents) which just fail to excite is exactly what one would expect on the basis of the Nernst-Hill theory. Further, they show that the frequency of an alternating current affects its efficiency, and the observed and calculated results agree closely.

Only one important result of theirs can be compared with ours. According to the theory, and according to the results obtained by many workers, stimuli of sufficiently short duration at any voltage produce no excitation. In our results, apparently, any duration of stimulus supplied at a voltage above a certain limiting value excites.

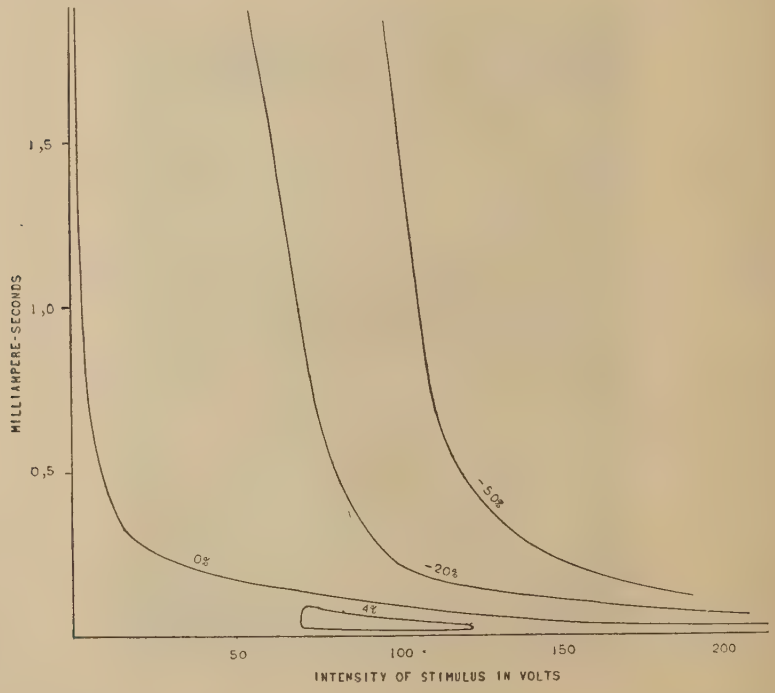

Fig. 9.

This marked difference of statement depends, perhaps, upon the definition of excitation. The animal physiologists consider that the nerve or muscle is excited if a propagated disturbance is set up, or a twitch initiated. In this work the excitation is measured, in a possibly more fundamental way, as a change in the permeability of the cell-membranes directly stimulated.

The theory, as it stands at present, can hardly explain the quantitative relations between the intensity and duration of the stimulus and the magnitude of the alteration in resistance; the fact that the permeability of a nerve or muscle possibly increases before "excitation" commences may compel re-investigation of the validity of the mathematical treatment as applied to nerve and muscle.

The results shown in fig. 7 have been used to calculate the quantities of current (in milliampere-seconds) at various voltages required to produce certain changes in resistance. These results are shown graphically in fig. 9, where the quantities of current are shown as ordinates, and the voltages at which they are

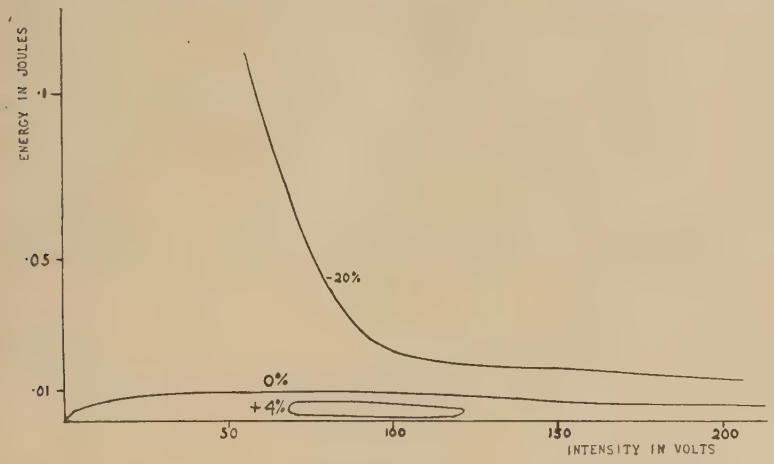

FIG. 10.

applied as abscissae. It appears that the higher the voltage the smaller is the quantity of current required to produce a given negative response; it is seen also that a given positive response (in this case + 4 per cent.) is obtained only within a rather limited range of voltages, no matter what quantity of current is

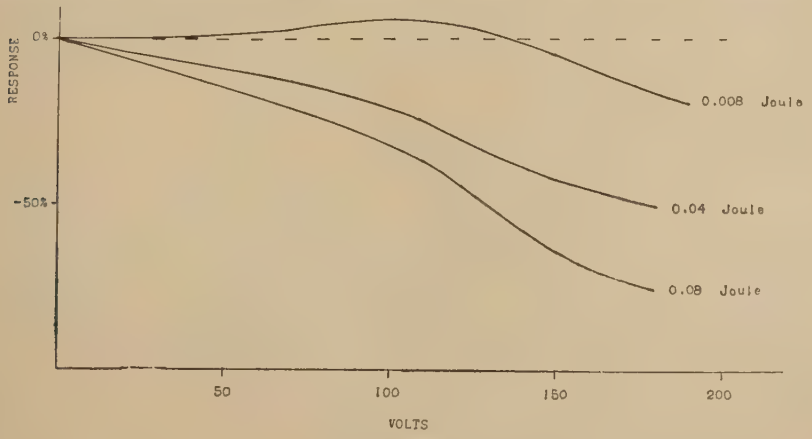

FIG. 11.

supplied. The nature of these relations is best understood from the curves of fig. 9.

These results have also been calculated so as to show the quantities of energy in joules (1 joule = 0·24 calorie) required to produce responses of

– 20 per cent., 0 per cent., and + 4 per cent. when the energy is supplied at voltages which are indicated on the horizontal axis (fig. 10). The energy-content of the stimulus has been calculated on the assumption that the inductance is negligible compared with the resistance.

There appears to be a voltage (70 volts) at which a maximum amount of energy is required to produce positive and negative reactions which cancel each other. This seems to be associated with the fact that positive reactions predominate over the negative only in a comparatively limited range of voltages, no matter what the energy content of the stimulus is. With stimuli after which negative reactions predominate, the energy required to produce a given response becomes smaller the higher the intensity at which it is supplied. These relations are seen in figs. 10 and 11.

The curves of fig. 11 show the responses produced by stimuli of definite energy contents (0·008, 0·04, and 0·08 joule) where the energy is supplied at various intensities. The S-shaped nature of the relation between the response and the intensity is clearly seen.

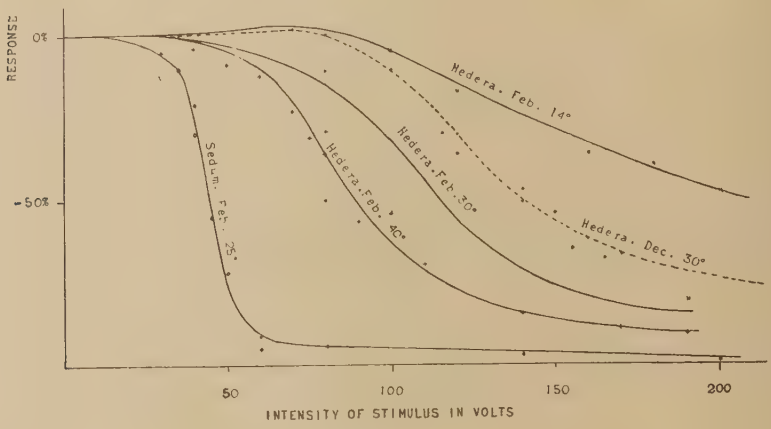

Fig. 12.

Influence of Season and Temperature on Sensitivity.

Since there is no quantitative measure of sensitivity, it must suffice at present to refer to four curves shown in fig. 12 and two in fig. 5. These illustrate the magnitudes of the responses obtained with stimuli of different voltages: in fig. 12 the time of stimulation was uniformly 0·8 sec. The broken curve embodies the results obtained in December, 1925, at 30° C.; the curve marked February 30° C. is from a similar set of experiments in February, 1926.

These, in conjunction with the curves of fig. 5, show the increasing effectiveness of a given stimulus as the season advances. The resistances of the leaves become smaller as the season advances (see fig. 2), but the very slightly larger currents flowing through the tissues in the later seasons are entirely inadequate to account for the increase in effectiveness of a stimulus of given voltage and duration.

This seasonal effect is to be distinguished from effects due to the age of the leaf. In very young leaves the number of membranes in a centimetre square is very much larger than in mature leaves, and these leaves have been

neglected. As the leaves get older, their resistance becomes greater; but in spite of the fact that smaller currents flow through these older leaves, in consequence of their higher resistance, the response of an old leaf to a stimulus of a given voltage is almost equal to that of a young leaf to a stimulus of the same voltage. The sensitivity, therefore, *appears* to increase slightly with age. To give an idea of the magnitude of the effect, the modal resistance of one-year-old leaves in March was 160,000 ohms, that of two-year-old leaves at the same date was 210,000 ohms.

The effects of temperature are seen in the three unbroken curves referring to the ivy in fig. 12. The results were obtained in the last week of January and the first fortnight of February. It should be mentioned that the behaviour of the leaves at 40° C. was somewhat erratic, and the curve drawn is of doubtful value.

The raising of the temperature causes, as one would expect, a marked increase in sensitivity; but it does not appear to affect all strengths of stimulus to a proportionate extent. Lack of sufficient data makes discussion of the cause of this differential effect impossible at present.

One other important external condition must be briefly mentioned: we have failed to observe any effect whatever of light on either the resistance or the sensitivity of ivy leaves.

The Effect of more than one Stimulus on the Response.

Lack of data again prevents any adequate discussion of the phenomena observed. Briefly, the facts are these: When a fairly small stimulus is given (e.g., 120 volts for 0·1 sec.), a response is obtained consisting of an alteration in resistance, and the effects do not disappear for about an hour after the stimulation.

A repetition of the same stimulus within this period causes a very slightly greater flow of current through the leaf-square owing to its lower resistance. This second stimulus causes a further drop in resistance, and the magnitude of this additional drop is about equal to that of the original one, when the second stimulus is applied within ten minutes of the first. If applied between the tenth and twentieth minute after the first stimulus, the second one is less than half as effective as the first. Later than half an hour after the first stimulus the sensitivity is again normal.

Although stimuli of given voltage and duration are really greater (i.e., consist of a greater quantity of current) if they are applied while the resistance is reduced in consequence of an earlier stimulus, yet the second stimulus is no more effective than the first, and, if applied at a period about 15–20 minutes after the first, it is much less effective.

Some interesting results were obtained by repeating stimuli too large to give positive and too small to give negative responses ("threshold" stimuli). It was found that the second "threshold" stimulus given shortly after the first brought about a response equal to that which a stimulus of twice the duration of the "threshold" would cause. If the interval between the two stimuli was increased beyond about 10 minutes, smaller responses were produced, and if the interval was as long as 20 minutes no response was produced.

The first "threshold" stimulus, therefore, has initiated some change or changes which only slowly disappear, although it has caused no change in resistance.

Changes in Resistance in Different Regions of the Stimulated Tissues.

Some results of experiments made with a view to finding out the extent to which the change produced by a stimulus affected different parts of the cells of tissues are of interest.

Firstly, it has been shown that when a current is passed through a piece of tissue longer than 1 cm., the voltage required to produce a given response is proportional to the length of the piece of tissue. The potential difference across each membrane is inversely proportional to the length of the piece of tissue.

A series of observations were made in an attempt to ascertain whether the change in resistance was less across the path of the stimulating current than along it. Centimetre squares of leaf were used on special electrodes. Two pairs of electrodes made contact, *via* Knop's solution, with the opposite sides of the square, and they were designed so that any of them could be disconnected and then reconnected with the leaf. This process was found to produce no change in the resistance as a result of an alteration of the area of contact.

The resistance was measured first across the square in one direction, then at right angles to this: these two resistances were almost always sensibly the same, unless a large vein ran across between two electrodes. The electrodes which were not being used were disconnected, as otherwise the current between the other electrodes was diverted from the leaf by them.

As the result of many experiments of this type, it has been found that the fall in resistance at right angles to the direction of the stimulating current was about 10 per cent. less than the fall in the direction of the stimulating current. The fall in resistance across the corners was about the same as the fall in the direction of the stimulus.

The potential gradient across the membranes (or the current density) is greatest in the tract directly between the electrodes, becoming slightly less as one approaches the edges of the leaf-square. The slightly lower transverse response is probably due to a slightly less stimulated zone at the edges of the leaf.

Pieces of leaves, 2 cm. by 1 cm., were also set up with two movable electrodes on each long side. These pieces were stimulated across one end, and the fall in resistance was measured in the stimulated region, and also parallel to the direction of the stimulus, at distances from the stimulating electrodes of 0·75 cm. and 1·5 cm. as measured from the centres of the electrodes.

In a typical experiment, the responses were as follows:—between the stimulating electrodes −26 per cent., at a distance of 0·75 cm. from a line joining them −10 per cent., and at a distance of 1·5 cm. −2 per cent. These figures may be explained as due to the spread of the current from the two electrodes through the rest of the conductor; but if they are due to the propagation of the excitation, they indicate a falling-off of the latter, unlike what is found in nervous tissue.

Attempts were made to explore the different regions of the conductor by using, in addition to the ordinary platinum foil electrodes, electrodes consisting of blacked platinum wires stuck through the leaf in certain places. The foil electrodes were at the ends of a long piece of leaf, 2·5 cm. apart, and the wire electrodes were used in pairs, each 0·5 cm. apart, at right angles to the line joining the foil electrodes. The resistance between the foil electrodes was less than that between the wire electrodes, this being probably due to the very small cross-sectional areas of the leaf in contact with the wires. Great differences in the resistances as measured between the different wires (from 300,000 to 500,000 ohms) were observed. This was probably, in part, due to differences in the contacts between them and the tissue.

The pieces were stimulated *via* the foil electrodes, and it was found that the responses as measured with different wires were in general different; for example, in one experiment the response between the foil electrodes was – 28 per cent.; between the wires of 3 successive pairs (– 47 per cent.), – 47 per cent., – 43 per cent., (– 42 per cent.), – 35 per cent., – 37½ per cent., (– 38½ per cent.). The figures in brackets are the responses at right angles to the direction of the stimulating currents. No relation was observed between the resistance between a pair of wires and the response between them.

This phenomenon still awaits explanation.

Responses in Sedum praealtum.

Before concluding, reference must be made to our examination of the effects of electric currents on the leaves of *Sedum praealtum*. Pieces of leaf 1 cm. long were used. The cross-sectional areas of different pieces were, however, very different; and on account of this the resistances of the pieces used varied very much, i.e., from 50,000 to 150,000 ohms. The experiments were carried out in January, 1926.

In one series of experiments four sets of leaves were put into the apparatus at 11 a.m., and their resistances were measured at intervals till midnight. The resistance rose to a maximum at about 5 p.m., and continued to fall after that till the experiment was discontinued at midnight. At its maximum value the resistance was 20–30 per cent. higher than it was at 11 a.m. and 10 p.m. At these times the resistances were equal.

This variation in resistance is probably associated with the very large diurnal variation in the concentration of acids in the cell-sap. The concentration is at its maximum at about 4 a.m., and at its minimum at about 4 p.m.

Leaves of low acid-content were used, and these were stimulated for 0·8 sec. The results of only one series of experiments are quoted, and, as before, the responses are plotted as ordinates and the voltages at which they were obtained as abscissae. This curve (marked Sedum, Feb., 25°) is seen in fig. 12.

It will be noticed that the curve is similar to the response-voltage curves of the ivy, but it is very much steeper, and the point of inflexion is at a very low voltage (43–44). The Sedum is, therefore, very much more sensitive than the ivy. In this connection it is of interest to note that since the cells of the Sedum are larger than those of the ivy, the potential difference per membrane is greater in the Sedum at a given voltage.

Discussion.

The method of studying the relation between stimulus and response described in this paper differs from those usually adopted in phototropic, geotropic, and thigmotropic researches, and in electro-physiology (2, 7), in the fact that the change constituting the response is observed in the stimulated cells themselves. The result of this is that the uncertainties connected with the propagation and the conversion of the propagated stimulus into the response are eliminated.

It has been found that the response to a stimulus takes time to develop. The maximum change in conductivity (i.e., permeability) is not attained instantaneously, but is only reached about 5 to 10 minutes after stimulation. This indicates that the changes cannot be entirely due to the concentration of ions in the neighbourhood of a membrane; for it is not likely that the change of concentration would continue to proceed for a sensible time after the stimulating current was cut off. It may be considered probable that the change in the concentration of ions produced by the stimulating current initiates a

secondary change in a colloid, which takes time to reach its maximum. This colloidal change may be supposed to affect the structure of the semipermeable membranes.

The resistance of tissues cannot logically be explained as due primarily to the back electromotive force of the ions accumulated at the membranes (polarisation). This back e.m.f. must itself be due to their mechanical structure.

That the resistance of tissues is principally due to the opposition to the passage of the electric current presented by the transverse protoplasmic membranes of the cells may be inferred from the fact that 90–99 per cent., according to the tissue, of the resistance is removed when the cells are rendered permeable. It is of note that the maximum of the positive reaction is attained later than that of the negative reaction.

Several stimuli applied within the interval of recovery evoke a response approximately equal to that evoked by a stimulus having the same intensity but having a duration equal to the sum of the durations of the successive stimuli. This gives additional evidence in favour of the view that the change brought about by the passage of a current is not merely due to the redistribution of ions. The colloidal change due to each stimulus persists for a measurable time, and hence the effect is cumulative.

The S-shape of the curves showing the relation between voltage and response is worthy of notice. This form of curve may be accounted for as follows: if the potential difference across a single membrane is less than some limiting value (which we will call the Minimal Potential), it may be supposed that the current does not effect any change in the resistance of the membrane. When this value is exceeded the change in this membrane, which the current effects, will depend upon the quantity of energy supplied irrespective of its voltage.

When a current makes its way across a leaf-square it passes along various tracks. In each of these tracks it traverses a certain number of membranes. The potential difference across each membrane will be inversely proportional to the number of membranes in each track, when a given voltage is applied. If these tracks were classified according to the number of membranes they contain, the frequency of occurrence of tracks, having very few and very many membranes, will be small, and the tracks containing the mean or modal number will be most frequent. Now when a sufficiently low potential difference is applied across the leaf-square the potential difference across the membranes of the tracks containing the fewest membranes will not exceed the minimal potential, and no change in resistance will result. As the potential difference across the leaf-square is increased, first, the minimal potential per membrane will be exceeded in the few tracks containing a small number of membranes. Then, with a continued rise in the potential difference across the leaf, the potential difference across the membranes will exceed the minimal potential in an increasing number of tracks, until the modal tracks are affected. From that point onwards for equal increases in the potential difference applied to the leaf-square the minimal potential per membrane will be reached in fewer and fewer additional tracks.

From this it will be plain that for equal increments in the voltage applied to a leaf-square, the increments in the number of tracks in which the minimal potential per membrane is attained will increase more and more rapidly up to a certain point (that at which the membranes of the modal track attain the minimal potential), after this point the increments in the number of tracks become successively smaller and smaller. This, of course, leads to an S-shaped relation between the number of membranes whose minimal potential is reached and the voltage applied to the tissue.

Two reactions, expressed as changes of resistance, are initiated in any membrane across which the minimal potential is applied. When the energy of the current is small the positive reaction predominates: increases in the amount of energy gradually cause the negative reaction to predominate. In each of these reactions, as the energy is increased, the magnitude of the alteration of resistance for a given alteration in energy gradually decreases. This decrease being more rapid in the case of the positive reaction than in that of the negative reaction, the limiting positive change is reached with a smaller energy of stimulus than is the case with the negative change: the limiting positive change is, moreover, of much smaller extent than the limiting negative change. The total response of a membrane will be the sum of these two changes. Hence the observed response attains a maximum positive value at a certain low energy of stimulation, beyond which it falls and changes to a negative response which finally attains a limiting value.

From this it appears that in considering the change of resistance of a leaf-square, we must take into account both the number of membranes affected, which will depend on the voltage of the stimulus—the relation between the two being expressed by an S-shaped curve, and also the nature and magnitude of the change in each membrane.

With long durations of current, and hence considerable quantities of energy, it is evident that any membrane across which the minimal potential is exceeded will respond to an extent approaching closely to its limiting response; hence the response of a tissue to a given voltage applied for a long time will be directly proportional to the number of membranes across which the potential difference has exceeded the minimal potential. The point of inflexion of such an S-shaped curve is round about 85 volts for 1 cm. sq. of Hedera leaf at 30° C. in February. It follows that when this voltage is applied to such a piece of leaf that the minimal potential will be attained across the membranes of the modal track. Since the number of cells across 1 cm. of leaf averages 80–100, we see that the minimal potential per cell is of the order of 1 volt.

The response-voltage curve of Sedum at long duration (fig. 12) is remarkable on account of the steepness of its region of inflexion and of the low voltage of its point of inflexion. This finds ready explanation on the hypothesis just described. The tissue of this leaf is much more uniform than that of Hedera. Hence the number of tracks, having approximately the modal number of membranes, form a larger proportion of the whole, and are responsible for the steep straight middle region of the curve. For it is evident that, if the tissue was absolutely uniform, the curve would become a vertical straight line. It is interesting further to note that the voltage of the point of inflexion is about 45 volts; and that the cells of the Sedum leaf have a diameter approximately twice that of those of Hedera, where the point of inflexion is at about 85 volts. The minimal potential per membrane is therefore of the same order in each of these leaves.

When shorter durations of current, and hence smaller amounts of energy, are used this simple relation is obscured by the fact that the membranes of tracks of high resistance respond positively. This pushes the whole response-voltage curve upwards and to the right.

The above considerations may be applied to the curves shown in fig. 13. Where 70 volts are applied to the leaf-square, the responses observed are the sum of the responses of the membranes of tracks having resistances below a certain value. The change in each membrane is of a dual character, viz., (1) a positive reaction developing rapidly for small increments of energy and reaching a limit while the energy is still small, and (2) a negative reaction developing less rapidly with increase of energy and attaining finally much

larger dimensions. The sum of these changes in the membranes involved will correspond to a curve similar to that shown in fig. 13, 70 volts.

When stimulating tissues with electric currents it is found that the quantity of energy does not alone determine the magnitude of the response, but that the intensity of the stimulus exercises as important an effect as its absolute quantity. From the preceding discussion it will appear that this controlling influence of the voltage is due to the fact that it decides the number of membranes responding. For a single membrane we may suppose that after a certain voltage has been exceeded only the amount of energy in the stimulus and not its voltage defines the nature and magnitude of the response.

This hypothesis seems to explain our observations, but, of course, further observations may decide in favour of some other view. For example, so far it has been found impossible to find a direct experimental test of the possibility that different voltages applied to a single membrane might evoke different responses.

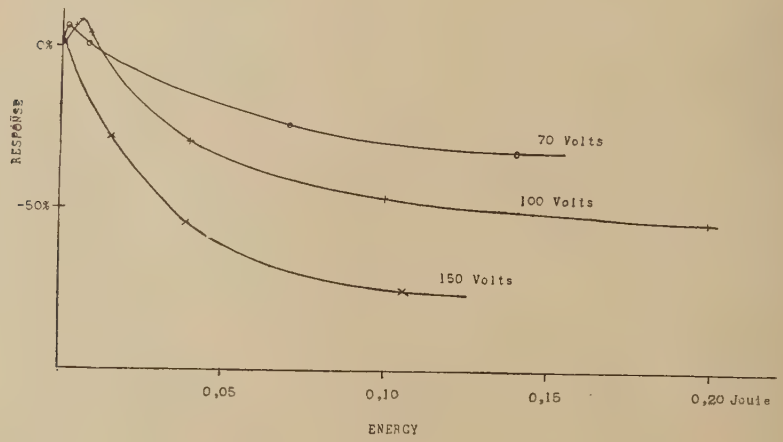

Fig. 13.

Summary.

The passage of an electrical current through a tissue leads to a change in electrical resistance and to a change in permeability. The quantitative aspects of the phenomenon have been investigated.

The stimulating current used was a sine-wave single-phase alternating current of 50 cycles, of which the voltage was controlled by a potentiometer, and the duration of the stimulus by a pendulum device. Pieces of ivy (*Hedera helix*) leaf, cut 1 cm. square, were used, and were found to maintain a nearly constant resistance in the apparatus for as long as three days in the absence of stimulus.

A moderate stimulus (say 120 volts for 0·1 sec.) is immediately followed by a very rapid fall in resistance, which becomes less and less rapid, and, after a few minutes, the resistance rises a little and falls again; finally it begins to rise slowly, recovering in about an hour to the value it originally had before stimulation.

Both a positive and negative deviation of resistance are initiated by the stimulus: and their relative magnitudes depend on the magnitude of the stimulus. The smallest possible current causes a response if a certain potential difference across the membrane is exceeded, and, as the stimulus is increased, at first the positive reaction increases the more rapidly and predominates over the negative; with still larger stimuli the negative reaction predominates to an increasingly great extent over the positive reaction, which finally becomes unnoticeable after very large stimuli.

It is shown that the positive reactions only predominate within a small range of intensities of stimulus, and also only when the energy content of the stimulus is less than 0·01 joule under the conditions of the experiments conducted in February.

Using stimuli, all of which are of the same energy content, it is found that the higher the intensity at which this energy is supplied to the tissue the more effective it is. The relation between the intensity and the response is S-shaped. In consequence of this, it is suggested that a given quantity of energy produces in any cell a definite alteration of resistance, whatever the voltage at which it is supplied. It is supposed that the ability of a current to stimulate a cell is determined by the potential difference across the membranes of the cell.

A series of stimuli applied to a single leaf-square evokes the same response as a single stimulus having a duration equal to the combined durations of the stimuli in the series, provided that the intervals between these stimuli are not too long.

Advance of the season and also rise of temperature greatly increased the sensitivity of the tissues: the positive and negative reactions were not affected to the same extent.

We desire to acknowledge a grant from the Dr. John Mallet Purser Endowment, which enabled us to procure much of the apparatus needed in this investigation, and also to express our indebtedness to Dr. J. H. J. Poole, who continually aided us throughout the work by putting his wide knowledge at our disposal. Dr. H. H. Poole has also helped us with many useful criticisms and suggestions.

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LIST OF FIGURES.

Fig. 1. General arrangement of apparatus.

A, Transformer for reducing voltage of supply for heating current; B, Voltmeter for measuring the voltage of applied current; C, Potentiometer for adjusting voltage of applied current; D, Contact Pendulum for timing the duration of the stimulus; E, Pohl's Commutator for introducing into circuit the applied current, or for introducing the tissue into one arm of the Kohlrausch Bridge; F and G, Two dial switches for introducing the pieces of tissue in succession into the arm of the Kohlrausch Bridge; H, Thermostat containing the pieces of tissue; J, Kohlrausch Bridge for measuring the resistance of the tissues; K, Battery and Buzzer for Kohlrausch Bridge; L, Test-tube in thermostat to receive electrodes and pieces of tissue; M, Electrodes to support pieces of tissue; N, Handle for raising the cork discs supporting the electrodes; O, Ratio-wire of Bridge; P, Sliding arm of Potentiometer, making contact with the rheostats; Q, Channel filled with mercury in which one contact of the sliding arm moves; R, Standard adjustable resistance in the Bridge; S, Contact past which the pendulum rubs when applying current; T, Telephone of Bridge; V, Bipolar switch for connecting this to transformer; W, "Zenith" resistances.

Fig. 2. Frequency of distribution of resistances (ivy-leaf squares).

Fig. 3. Time course of responses.

Fig. 4. Frequencies of distribution of responses to a stimulus of 120 volts for 0·1 sec.

Fig. 5. Relations between responses and intensities of stimuli of various durations.

Fig. 6. Relations between responses and the durations of stimuli.

Fig. 7. Intensities and durations of stimuli required to produce certain definite responses.

Fig. 8. Solid graph to show responses evoked by stimuli of various voltages and durations.

Fig. 9. Quantity of current required to evoke certain responses at various voltages.

Fig. 10. Quantity of energy required to evoke certain responses at various voltages.

Fig. 11. Responses evoked by certain quantities of energy supplied at various intensities.

Fig. 12. Response-voltage curves for Ivy and Sedum at various temperatures.

Fig. 13. Relation between responses and quantities of energy supplied at certain intensities.

No. 30.

CANINIA CYLINDRICA SCOULER AND OTHER LARGE *CANINIAS* FROM THE CARBONIFEROUS LIMESTONE OF IRELAND.

By HERBERT P. LEWIS, M.A., F.G.S.,
Lecturer in Palæontology in the University of Sheffield.

(Plates XVI and XVII.)

[Communicated by Dr. L. B. Smyth.]

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I.—Introduction.

In this paper are described certain corals of large size belonging to the genus *Caninia*. The majority of these are now preserved in the Geological Museum of Trinity College, Dublin, and were collected from the Carboniferous Limestone of Co. Donegal, Co. Roscommon, Co. Sligo, and Co. Tyrone.

For the loan of these specimens, and for allowing me to cut sections of them, I am much indebted to Professor Joly. Dr. L. B. Smyth has made a long-desired addition to our knowledge of *Caninia* by cutting a section, and writing a full description of the type-specimen of *Caninia* (*Siphonophyllia*) *cylindrica* Scouler. Our thanks are due to the Acting Director and to Mr. A. W. Stelfox, of the National Museum of Ireland, Dublin, for the loan of the specimen for this purpose.

Specimens from the Carboniferous Limestone of N. Wales and the Morecambe District of the North-West Province are figured and described in order to show their relationship to *Caninia benburbensis* sp. nov.

The Irish specimens were collected many years ago, and, unfortunately, no more than the barest information is available concerning the localities from which they were obtained. It is also to be regretted, although not surprising, that only the adult or ephebic portions of the coralla are represented, and their ontogeny cannot, therefore, be investigated. These specimens are, however, so much better preserved than is usually the case with such large corals, that figures and descriptions of the adult features they present, especially those seen in transverse and longitudinal sections, should be put on record, in order that comparison may be made with other similar corals from known geological horizons.

II.—*CANINIA*. H. Michelin.

Caninia: H. Michelin, 1840. Congrès de Turin.
Siphonophyllia: Scouler, 1843. Syn. Char. Carb. Foss. Ireland, by F. M'Coy.
Caninia: A. Salée, 1910. "Le Genre *Caninia*."
Genotype: *Caninia cornucopiae*, Mich., 1840, *op. cit.* (See R. G. Carruthers, 1908. Geol. Mag., p. 158.) Tournaisian. France and Belgium.

Diagnosis.—Simple. Rugose corals, turbinate and conical, often cylindrical for a great part of their length.

Major septa well developed, but usually not meeting at the centre in the adult cylindrical portion of the corallum. Minor septa of various lengths in

different species. Cardinal fossula conspicuous and "open," i.e., limited only by tabulae at the inner end. Tabulae well developed, but variable in regularity: they may be arched and "vesicular." Dissepiments are typically few, but in advanced species are numerous, forming a wide extrathecal zone.

III.—*CANINIA CYLINDRICA* Scouler. Plate XVI, Figs. 1*a* and 1*b*.

Holotype: *Caninia* (*Siphonophyllia*) *cylindrica* Scouler. — Griffith Coll., National Museum of Ireland, Dublin. No. "80–1925." Arenaceous Limestone Ardsallagh, Drumquin, Ireland. (See A. Salée, "Le Genre *Caninia*." Brussels, 1910, p. 31, and for synonomy, pp. 27–28.) Ref. F. M'Coy, "Synopsis, Char. Carb. L'stone. Foss. Ireland," Dublin, 1844,[1] p. 187 and Pl. 27, fig. 5. (The figure is reversed.)

Description of the Prototype by Dr. L. B. Smyth.

1. *Characters revealed by weathering.* Pl. XVI, fig. 1*a*.—"The specimen is embedded in limestone, so that the external surface is nowhere exposed. A rough longitudinal section has been made by weathering, so that less than half the cylinder is present. Both ends are missing. The portion preserved has a total length of 14 cm., of which 4 cm. consists of the dissepimental wall only. The specimen is strongly silicified, and weathering has etched it out considerably.

"The diameter, as far as can be judged from the weathered surface, varied from 6 to 7 cm., about 3·5 cm. being intrathecal; the remaining variable part being dissepimental zone. Much of this zone seems to be absent. M'Coy's figure is restored, and gives a false impression of the regularity and completeness of the epitheca.

"The tabulae extend entirely across the intrathecal space. They are flat, or gently concave upwards in the middle 2 cm. of their diameter, but bent strongly downwards at their outer edges. They are mostly complete and separate, but occasionally two run together at one or both ends of a cross section. They have an average spacing of six tabulae in a length of 1 cm.

"There is a deep, funnel-like, fossular depression at the edge of each tabula, those in successive tabulae fitting into one another. The fossula reaches farther towards the centre of the tabula than do the adjacent major septa.

"The major septa are vertically continuous for about 7–8 mm. inwards from the theca, i.e., in the zone in which the tabulae are bent downwards. Farther towards the centre they become detached from the under surfaces of the tabulae, and their upper edges descend rapidly, until the septa die out on the upper surfaces of the tabulae, leaving free of septa a space of about 13 mm. diameter in the middle of the tabulae.

"The interdissepimental spaces are large and irregular. Septa may be seen within them as very slightly elevated ridges."

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[1] The species *Siphonophyllia cylindrica* Scouler MS. I find first named in the table of fossils which forms the bulk of a *Notice respecting the Fossils of the Mountain Limestone of Ireland as Compared with those of Great Britain and also with the Devonian System*, by Richard Griffith, 1842, Dublin, p. 9. No description accompanies the name, but the species is recorded as occurring in the "Mountain Limestone of Ireland," in the "Yellow Sandstone" of the "Northern District," the "Carboniferous Slate" of the "Middle and Northern Districts" (abundantly in the latter), in the "Lower Limestone" of the "Southern and Northern Districts," and, very abundantly, in the "Calp" of the "Northern Districts." It is probable that these records do not in every case refer to *C. cylindrica* Sc. s. str.

2. *Internal characters as seen in transverse section.* Pl. XVI, fig. 1*b*.—"The section was cut near the proximal end of the specimen. It consists of about one half of a complete section, and contains one cardinal quadrant complete, the greater part of the other, and a portion of one counter quadrant. The greatest diameter of this section is 5·8 cm., but, owing to its silicified state, it is a little doubtful if the epitheca is present at one end of this line of measurement. The width of the dissepimental zone reaches 1·4 cm. The interdissepimental spaces are irregular and very unequal; the largest appear to occur a little way in from the epitheca, thence they get, on the whole, smaller inwards.

"Projecting from the dissepiments are tooth-like sections of septal ridges. These may be absent from some of the outer dissepiments; they become longer in general as the theca is approached, but they do not join up to form continuous septa outside the theca. The intrathecal region has a diameter of 3·5 cm. It contains 29 major septa, which appears as if it might be one half the total number, or perhaps a little more than half. They extend about 9 mm. inwards from the theca, and are radially disposed without any curvature relatively to the fossula. Between them are minor septa projecting inwards about 2 mm. from the theca. All the septa and the thecal wall are slightly thickened, the thickening becoming somewhat greater as the fossula is approached. The cardinal septum and the septa bounding the fossula are strongly thickened, the stereoplasm being thickest at a little distance from the theca, and thence tapering gradually to a point. Four or five tabulae are intersected by the section."

Remarks.—In his review of Professor Salée's memoir, "Le Genre *Caninia*," Mr. R. G. Carruthers pointed out in the Geological Magazine in 1911 (p. 321) that "the weak spot of the revision lies in the question of nomenclature between *Caninia cylindrica* and *Caninia gigantea*" (Michelin, *Iconographie Zooph.*, pp. 81 and 255; Pl. 16, figs. 1*a*, 1*b*, 1*c* (? coeteris), 1840–1847), and that "if it can be said that Michelin's type is lost, and that similar material from Tournai varies too much to be used as a basis for redescription, while the topotypes of M'Coy's species are constant in character and agree with Salée's account of his Tournai material, then the revision can be accepted as a most satisfactory piece of work. But in the meantime the question remains an open one."

Dr. Salée, from an inspection of a cast of the holotype of *Caninia cylindrica* Scouler, was convinced that it represented the type of his Tournai material. Comparison of the foregoing description by Dr. Smyth and the figured section accompanying it, with Dr. Salée's description and figures of his Belgian material, upholds in every way Salée's opinion, and in no way detracts from its accuracy.

IV.—*CANINIA* cf. *CYLINDRICA* Scouler. Pl. XVI, Figs. 2*a* and 2*b*.

Distribution.—Lower Carboniferous Limestone, Carrowgarry, Co. Sligo.

1. *External Characters.*—The length of the known, straight, cylindrical portion of the corallum is 14 cm. and the maximum width 6·5 cm. There are alternating annular growth-swellings and constrictions (about five swellings in 3 cm.). The matrix is a crinoidal limestone, and the external surface is "beekitised" to such an extent that the finer growth lines on the epitheca are destroyed.

The distal end is weathered, and shows septa and dissepiments much silicified and minute quartz crystals on the middle tabulate portion. The fossular depression is discernible. Calyx unknown.

2. *Internal Characters.* (*a*) *Transverse sections.* Pl. XVI, fig. 2*a*. — The epitheca is thin. There are 70 major septa at a diameter of 6·2 cm., at which the width of the dissepimental zone is 1·25 cm. Minor septa are absent. The major septa show stereoplasmic thickening, especially in the cardinal quadrants. They are continuous for some distance intrathecally, but only for a short distance extrathecally, for in the peripheral portion of the dissepimental zone the septa project as teeth from the dissepiments, exactly as in *C. cylindrica.* The cardinal fossula is well marked by the shortening of the cardinal and adjacent septa, and by the curved intersections of the tabulae, which are steeply depressed in this region. The counter fossula is not very conspicuous.

The outer portion of the dissepimental zone is made up of large curved dissepiments, but, near the theca, as the major septa become continuous, these give place to dissepiments, usually of "herring-bone"[1] type. The theca is to some extent thickened by stereoplasm.

(*b*) *Longitudinal sections.* Pl. XVI, fig. 2*b*. — A sharp division exists between extra- and intrathecal regions. The theca itself is thickened, especially on the cardinal side. In sections through both cardinal and counter fossulae there are about 9 tabulae in a length of 1 cm. Of these only a few extend from the theca on the counter side to the "siphonal" depression at the cardinal fossula. The tabulae, as a whole, are flat in the middle, normally somewhat depressed to the theca at their edges, and sharply deflected into a vertical, or almost vertical, position at the cardinal fossula. In this way is formed a distinct "fossular depression" some 5 mm. wide, bounded on the outer side by the innermost dissepiments, which are thickened on their convexities and jointly constitute the theca, and on the inner side by the down-turned continuations of the main tabulae. Crossing the fossular or "siphonal" depression are *fossular tabellae,* which arise from the upper or outer sides of the main tabulae, sag somewhat proximally, and then, usually, rise to join the theca.

The majority of the dissepiments are long, and arranged in rows which dip steeply near the theca, and tend to make a smaller angle with the horizontal as they approach the epitheca.

Remarks.—Practically the only difference which exists between this coral and *C. cylindrica* Scouler, forma typica, is the absence of minor septa in the former, and their presence as projections into the intrathecal region in the latter. In Dr. Salée's figures in "Le Genre *Caninia*" of longitudinal sections of *C. cylindrica,* the "siphonal" depression is by no means so well displayed as in the section figured on Pl. XVI (fig. 2*b*). This feature may be considered by some authorities of sufficient importance to necessitate a return to the generic name *Siphonophyllia* for the larger corals of *cylindrica* type. Even if difference in fossular character were of generic importance, in the present case there is no essential difference between the fossular depression of *Caninia cornucopiae,* the genotype of *Caninia,* and that of corals of the *C. cylindrica* group; consequently the name *Siphonophyllia* cannot be restored on the strength of fossular difference only, as suggested by Miss O'Connell in her paper[2] concerned with the generic nomenclature of *Siphonophyllia, Caninia,* and *Zaphrentis,* for no fossular difference exists between *Caninia* and "*Siphonophyllia.*"

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[1] See Lewis, Quart. Journ. Geol. Soc., 1924, 80, pt. 3, pp. 402 and 403, fig. 2, for an explanation of this dissepimental pattern. In the present paper I use the word "concentric" in preference to "rectangular" employed in the 1924 paper to describe another dissepimental arrangement (p. 403).

[2] New York Acad. Sci., 1914, 28, pp. 177–192.

V.—*CANINIA* sp. Pl. XVI, Figs. 3*a* and 3*b*.

Distribution.—Carboniferous Limestone, Donegal (West Coast).

1. *External Characters.*—This species is represented in the collection of Trinity College Museum by two individuals presented by Francis Olpherts, Esq. The locality given is extremely vague. Attached to both specimens are corallites of *Aulopora*, and both appear to have been subjected to similar mineralogical changes, silicification, and replacement of calcite by barytes. Possibly, therefore, they were obtained from the same beds.

One specimen consists of about one half of a slightly curved corallum. This has been weathered, so that a rough longitudinal section 15 cm. long and 7 cm. wide is exposed. The other specimen is straight and cylindrical. It has a length of 20 cm. and a greatest diameter of 6 cm. The main growth-swellings recur about every 2 cm., and swellings and constrictions merge into one another, so that in longitudinal section the epithecal outlines are as shown in Pl. XVI, fig. 3*b*. On the other hand, the growth-swellings may end abruptly, and then there is an appearance of closely repeated rejuvenation of the dissepimental zone at somewhat irregularly spaced intervals. Both coralla are encircled by finer ridges, of which there may be as many as five in 1 mm.

The intertabular spaces are usually infilled with barytes. In one specimen (see Pl. XVI, fig. 3*b*) this appears to have resulted in the destruction of some of the shorter tabulae, so that in this specimen there are only 4 or 5 tabulae in a vertical distance of 1 cm., while in the other there are 7 or 8 in 1 cm.

Where the epitheca has been worn thin or wholly removed, the major septa project as longitudinal ridges. Although the dissepimental tissue of one specimen is exposed in several places, the epitheca of the other has remained remarkably intact, notwithstanding the silification and barytisation of the interior. The calyx is unknown.

2. *Internal Characters.* (*a*) *Transverse sections.* Pl. XVI, fig. 3*a*.—In a section about 6 cm. in diameter there are 72 major septa, all thickened by stereoplasm to about the same extent. The middle portion of the tabulate area is composed chiefly of barytes, secondary silica, and recrystallised calcite; consequently the distal ends of the septa are usually destroyed. The major septa are continuous across the dissepimental zone. The minor septa are spasmodic in their occurrence. They are discontinuous, and are found projecting as small teeth from the inner side of the dissepiments. They are destroyed by silicification to some extent. The cardinal septum is shorter and the counter septum longer than those major septa which are respectively adjacent to each.

Between the tabulae there is much barytes, and the tabulae themselves have provided surfaces along which recrystallisation has occurred. A cardinal fossular depression extends some 1·5 cm. inwards from the theca. The counter fossula is distinguishable.

The extrathecal zone is built up of dissepiments arranged concentrically, i.e., more or less parallel to the epitheca. This "concentric" type is consequent on the continuity of the septa across the dissepimental zone, while on the failure of the minor septa depends to a great degree the occurrence of the "herring-bone" type.

(*b*) *Longitudinal sections.* Pl. XVI, fig. 3*b*.—In the intrathecal region only the main tabulae appear to be preserved, the shorter and finer ones having been destroyed. About 4 tabulae are now seen in a height of 1 cm. They

are almost flat in the middle, but, in sections cut normal or obliquely to the plane passing through cardinal and counter fossulae, a pronounced sag can be seen in the middle portion of the tabulae. The tabulae plunge rather steeply before joining the theca, which is thickened. The width of the tabulate area is 3·75 cm.

The dissepiments composing the extrathecal region, which is 8 mm. wide, are arranged in almost vertical rows. The interdissepimental spaces are in the main of large size.

Remarks.—This species most closely resembles a form which occurs somewhat abundantly in the Carboniferous Limestone on the foreshore near Strandhall in the Isle of Man, associated with fossils characteristic of the Lower Dibunophyllum zone (D_1).

VI.—*CANINIA BENBURBENSIS* SP. NOV. PL. XVI, FIGS. 5 AND 6, AND PL. XVII, FIGS. 1*a*, 1*b*; 2*a*, 2*b*; 3*a*, 3*b*, 3*c*, AND 4.

Synonymy. — *Caninia* cf. *samsonensis.* Salée. (Lewis, in L. B. Smyth: "A Contribution to the Geology of Great Orme's Head," Scient. Proc. Roy. Dub. Soc., 1925, **18**, No. 12, pp. 145–6).

Diagnosis. — Simple Rugose corals with no columella or central column. Major septa extending well into the intrathecal tabulate region, and usually continuous across the entire width of the wide dissepimental zone. Minor septa generally show some continuity extrathecally near the theca. A well-marked cardinal fossula is present, and a counter fossula is also conspicuous.

Distribution.—Carboniferous, Visean, Dibunophyllum zone. In N. Wales and the Morecambe district the species is found at horizons in D_1 and D_2 beds not far removed from the base of D_2. In Ireland the species, including the holotype, occurs in beds of unknown horizons at Benburb (Co. Tyrone).

Holotype. — No. T96/1010, Trinity College, Dublin (Geol. Museum) Carboniferous Limestone, Benburb (Co. Tyrone). The characters at the ephebic stage are alone known, and sections at this stage are figured on Pl. XVII, figs. 1*a* and 1*b*.

1. *External Characters.* — All specimens examined represent the distal, cylindrical or slightly curved portions of simple coralla. Of these the largest was 28 cm. long and at its widest 8 cm. in diameter. The calyx is not known.

When unworn the epithecal surface is smooth, or encircled by somewhat inconspicuous striations. Removal of the epitheca brings into prominence the ends of the septa as longitudinal ridges. In addition to the annular striations, growth-swellings and constrictions on a larger scale occur, and are caused by the widening and narrowing of the dissepimental zone, the intrathecal cylinder maintaining a constant diameter.

Weathering has occasionally exposed the under surface of a tabula, showing it to be, on the whole, flat, but a fairly deep depression, some 5 mm. wide and commencing about 8 mm. from the centre, marks the cardinal fossula. Elsewhere the tabular margin is slightly depressed. The sagging of a tabula between the septa which rest on its upper surface produces corrugations which extend inwards radially from the theca for 6 mm. Where the upper surface of a tabula is exposed the septal prolongations on its surface are seen to extend to a greater distance from the theca than this.

When the corallum is slightly curved, and so placed that the convexity is away from the observer, the cardinal fossula and the more greatly thickened septa may be situated towards the right-hand side.

2. *Internal Characters.* (*a*) *Transverse sections.* Pl. XVI, figs. 5 and 6, and Pl. XVII, figs. 1*a*, 2*a*, 3*a*, 3*b*, and 4.—The epitheca is thin. For a diameter of the corallum of from 7·5 to 8 cm., or of the intrathecal cylinder of 4·5 to 5 cm., the number of major septa is about 74. In narrower (? younger) individuals there are 60 major septa when the width of the tabulate region is 3·5 cm. The major septa are straight for the greater part of their length in the tabulate area, the middle of which they do not reach, but more or less sinuous in the dissepimental zone. Within the tabulate area all the major septa are equally, and sometimes strongly, thickened by stereoplasm (see Pl. XVII, fig. 1*a*), and taper inwards. The minor septa are practically confined to the extrathecal area, but in some forms they project a short distance into the intrathecal area. In the dissepimental zone the minor septa usually extend from the theca in a somewhat disjointed manner for about one half, or two thirds, the width of the zone. Near the theca their continuity is more marked, but diminishes as the epitheca is approached. The cardinal fossula is well-marked and fairly long, and appears wide or narrow according to the plane in which the section cuts the tabulae. One short major septum extends into it. The counter fossula is usually well-marked (see Pl. XVI, fig. 6, and Pl. XVII, figs. 2*a* and 3*a*), and like the cardinal fossula is of "open" type, i.e., not closed by septa at its inner end.

Extrathecally the interseptal spaces are partitioned by dissepiments tending towards the "herring-bone" arrangement. Dissepiments of "concentric" type are also present. Where minor septa occur, the dissepiments become more numerous and crowded between major and minor septa. These smaller dissepiments are of "concentric" type, the "herring-bone" pattern being destroyed, or rather replaced, by the advent of minor septa. The width of the dissepimental zone varies from 1 to 1·5 cm. The inner ring of dissepiments forms the theca, which is thickened by stereoplasm.

(*b*) *Longitudinal sections.* Pl. XVII, figs. 1*b*, 2*b*, and 3*c*.—Extending across the sections from theca to theca are some 3 or 4 main tabulae in a vertical distance of 1 cm. These are thickened, and show a flattened or sagging middle portion, 2 cm. to 2·5 cm. wide. They have a sharp deflection to the theca. Between the main tabulae occur from one to four subsidiary and less thickened tabulae, along the upper surfaces of which the pennant-like projections of the major septa do not extend for so great a distance as they do along the upper surfaces of the main tabulae. In sections through cardinal and counter fossulae the deflections of the tabulae, at a distance of some 5 mm. from the theca, into a vertical or almost vertical position, and their invagination marks the "fossular depression" at the cardinal fossula. This "siphonal" or fossular depression is crossed by fossular tabellae, which in longitudinal sections sometimes appear funnel-shaped (see Pl. XVII, fig. 3*c*).

Within the extrathecal region are rather elongated, but frequently irregularly sized, interdissepimental spaces, arranged in rows, making a high angle with the horizontal. The theca is thickened by stereoplasm, and forms a distinct wall.

Variations of Caninia benburbensis.

Although a certain definiteness must of necessity be lacking when the earlier developmental stages in the life-history of the individuals constituting a species are unknown, yet it is possible, pending the discovery of better material, to gain some conception of the structural changes which the species has undergone by a study of various individuals showing adult characters only. In *C. benburbensis*

the following variations are recognizable, and give evidence of anagenesis and catagenesis during the life-history of the species :—

A.—*The Holotype,* already described (see above and Pl. XVII, figs. 1*a* and 1*b*), may be regarded as the central type, representing the species at its acme. The specimens from Great Orme's Head are closely related to the holotype (see Pl. XVI, fig. 5).

B.—*Transitional forms* (Pl. XVI, fig. 6, and Pl. XVII, fig. 4).—These are recognised in corals from the "North-West Province" of the English Carboniferous Limestone (i.e. from Humphrey Head, near Grange,[1] and Silverdale, near Arnside). They show characters reminiscent of *C. cylindrica,* in that the peripheral region of the dissepimental zone is composed of large curved dissepiments, and that the minor septa advance some distance into the intrathecal region. Some of the characters distinguishing them from *C. cylindrica* are the crowding of the minute dissepiments near the theca, and the tendency for both major and minor septa to be continuous, especially near the theca, in the extrathecal region.

In 1906[2] Dr. T. F. Sibly figured a transverse section of a coral which he regarded as typical of what he termed the "Caninoid Cyathophylla." Dr. A. Salée has since included this form in his synonymy of *C. cylindrica* Scouler.[3] This coral from the C. beds of the Mendips so strongly resembles the Humphrey Head form (see Pl. XVI, fig. 6) in several respects that there is little doubt that the ancestry of *C. benburbensis* is to be sought among the earlier variants of *C. cylindrica,* of which Dr. Sibly's form is typical.

C.—*Senescent forms* (Pl. XVII, figs. 2*a*, 2*b*, 3*a*, 3*b*, and 3*c*).— Two of the Benburb specimens show characters which can best be explained as indications of catagenesis in the life of the species. Not only have the major septa become sinuous, but they are usually split along their middle lamella; the halves have separated, and individually become gnarled and twisted. The minor septa do not show their normal continuity, and, in sections at an early ephebic stage, are sometimes difficult to detect. Stereoplasmic thickening is irregular, and while this may affect some of the dissepiments, the cardinal septum is at times practically unthickened.

The coral exhibits marked rejuvenescence. Not only does the dissepimental zone vary in width, but at intervals of a few inches the corallum, after attaining the maximum width and exhibiting characters fully expressive of old age, suddenly contracts, so that a reduction of 1 cm. in the width of the intrathecal cylinder may take place without diminution in the number of septa. The septa are more regular and thinner in the rejuvenated portion, and the cardinal and counter fossula remain conspicuous.

These forms indicate that the species had reached senility and was about to suffer extinction.

VII.—*CANINIA* cf. *PATULA* Mich. Pl. XVI, Figs. 4*a* and 4*b*.

Distribution.—Carboniferous Limestone. Cleene (Co. Roscommon).

1. *External Characters.*—The corallum, which has a maximum diameter of 4 cm., and is curved towards the proximal end, is cylindrical for the greater part of its length, which attains 24 cm. With the convexity of the coral away from

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[1] E. J. Garwood, "Lower Carb. Succession in the North-West of England." Q.J.G.S., 1912, 68, p. 530, records this form as "*Caninia* cf. *cylindrica* Sc.," and on p. 513 records the Silverdale form from Trowbarrow Quarry as a "Caninoid *Campophyllum.*"

[2] Q.J.G.S., 1906, 62, p. 367, Pl. XXI, fig. 1.

[3] "Le Genre *Caninia.*" Brussels, 1910, p. 28.

the observer, the cardinal fossula and more greatly thickened septa are on the left-hand side. In the adult cylindrical portion constrictions recur every 1·5 to 2 cm. The epithecal surface is smooth, although encircling striae may occur about 1 mm. apart. Longitudinal ridges are only prominent when the epitheca is worn. The calyx is unknown.

2. *Internal Characters.* (*a*) *Transverse sections.* Pl. XVI, fig. 4*a*. — The epitheca is fairly thin. There are 60 major septa at a diameter of 4 cm., and those in the cardinal quadrants are the more greatly thickened. The major septa extend to the middle of the intrathecal region. Those adjacent to the cardinal fossula have their distal ends curved towards the inner side of the fossula, which is broad and conspicuous. The cardinal septum is short. Minor septa extend inwards from the epitheca across about four rows of dissepiments, and attain a length of 2 mm.

The inner ring of dissepiments is not thickened, and consequently the "theca" is not conspicuous. The extrathecal region is about 7·5 mm. wide, and is made up of dissepiments often arranged "concentrically" near the theca, but tending towards the "herring-bone" pattern farther out.

(*b*) *Longitudinal sections.* Pl. XVI, fig. 4*b*.—In the intrathecal region there are about nine tabulae in a length of 1 cm. The tabulae are seen to have a steeper dip to the cardinal than to the counter fossula in a section through both fossulae. The dissepiments are variable in size, and arranged in rows which are steeply inclined towards the theca.

Remarks.—In the specimen figured (Pl. XVI, figs. 4*a* and 4*b*) it is evident that fracture, followed by crushing and movement along the fracture plane, and, finally, by recementation, have obscured part of the structure. The characters which the coral exhibits are nevertheless sufficiently definite to warrant its inclusion in the group recognized by Dr. Salée as *C. patula*, Mich. It agrees most closely with the *tortuosa* type of that species which is to be found, for instance, in the γ beds of Barry Island, S. Wales.

In conclusion, I wish to thank Dr. L. B. Smyth for his kindness in supplying me with the bulk of the material here described, and for his valuable contribution to this paper. To Professor E. J. Garwood, F.R.S., I am indebted for allowing me to section the specimen of *C. benburbensis* from Humphrey Head.

EXPLANATION OF PLATES.

Plate XVI.

All figures, except fig. 1*a*, are of natural size.

Fig.

1. *Caninia cylindrica* Scouler (M'Coy). Prototype No. "80–1925." Griffith Coll., Nat. Museum of Ireland. Carboniferous Limestone, Ardsallagh, Drumquin, Ireland: (*a*) weathered longitudinal section × ½; (*b*) transverse section. Sectioned and photographed by Dr. L. B. Smyth.

2. *Caninia* cf. *cylindrica* Sc. No. F93/1010, Lower Carboniferous Limestone, Carrowgarry, Co. Sligo: (*a*) transverse; (*b*) longitudinal section.

3. *Caninia* sp. No. F94/1010, Carboniferous Limestone, Donegal (West Coast), presented by F. Olpherts: (*a*) transverse; (*b*) longitudinal section.

4. *Caninia* cf. *patula*, Mich. No. F99/1010, Carboniferous Limestone, Cleene, Co. Roscommon: (*a*) transverse; (*b*) longitudinal section.

5. *Caninia benburbensis* sp. nov. No. F100/1010, Carboniferous Limestone, Visean, D_2 beds, "Caninia Band," Great Orme's Head, Llandudno, N. Wales. Collected by Dr. L. B. Smyth. Transverse section.

6. *Caninia benburbensis* sp. nov. Geol. Museum, University College, London. Lower Lonsdaleia Beds (Base of D_2), Humphrey Head, near Grange, Lancashire. Collected by Professor E. J. Garwood. Transverse section.

Plate XVII.

The figures (excepting fig. 4) are of corals from the Carboniferous Limestone of Benburb, Co. Tyrone, presented by W. Murray, and all are of natural size.

Fig.

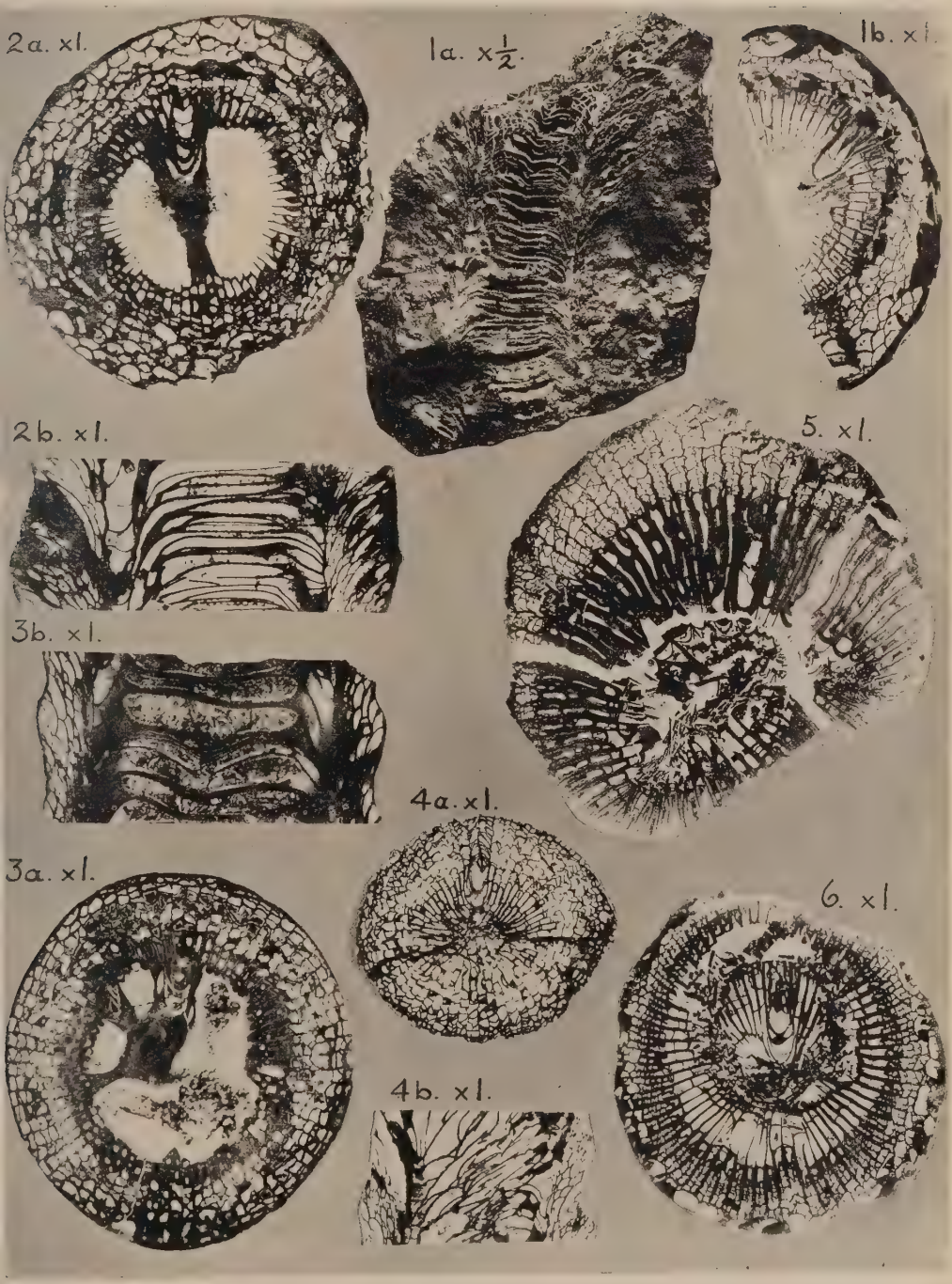
1. *Caninia benburbensis* sp. nov. Holotype. No. T96/1010, Trinity College, Dublin (Geol. Museum): (*a*) transverse; (*b*) longitudinal section.

2. *Caninia benburbensis* sp. nov. No. F97/1010, Catagenetic form: (*a*) transverse; (*b*) longitudinal section.

3. *Caninia benburbensis* sp. nov. No. F98/1010, Catagenetic form (a younger specimen): (*a*), (*b*) transverse sections; (*c*) longitudinal section.

4. *Caninia benburbensis* sp. nov. No. F95/1010, Early form. Nodular Bed (near top of D_1), Trowbarrow Quarry, Silverdale, Lancashire, transverse section.

The register numbers refer to specimens in the Geological Museum, Trinity College, Dublin, where, unless otherwise stated, the specimens and sections are now preserved.


2a. x1.
1a. x½.
1b. x1.
2b. x1.
5. x1.
3b. x1.
4a. x1.
3a. x1.
6. x1.
4b. x1.

1a. ×1.
1b. ×1.
2b. ×1.
2a. ×1.
4. ×1.
3c. ×1.
3a. ×1.
3b. ×1.

No. 31.

STUDIES ON PEAT. PART II. DISTILLATION UNDER REDUCED PRESSURE OF CERTAIN CONSTITUENTS OF PEAT.

By JOSEPH REILLY, M.A., Sc.D., and JOAN SULLIVAN, M.Sc.

[Read December 21, 1926. Published separately February 14, 1927.]

Introduction.

In Part I of this work (Reilly and Pyne, Sc. Proc. R.D.S., 1926, **18**, No. 27, 329), it was shown that the decomposition products from the distillation of peat, even under reduced pressure, were very complex. To simplify the conditions of the experiment it was considered desirable to separate the peat, in the first instance, into its various constituents, and to investigate the distillation products of these separately. Though many investigators have fractionated peat, principally for the preparation of humic acid, no attempt appears to have been made to subject these various fractions to distillation under reduced pressure.

I.—*Fractionation.*

The peat, similar to that previously employed, was air-dried by spreading it out in thin layers and exposing it to the room temperature for about a fortnight. This air-dried peat was then ground to a fine powder, and dried at 100° C. until a constant weight was obtained. As peat in this condition is very hygroscopic, it was kept in stoppered vessels until required for use.

The procedure followed in separating the peat into its constituents was very similar to that used by earlier workers (cf. Beckley, J. Agric. Sci., 1921, **11**, 66). The anhydrous, finely-powdered peat was extracted with dilute hydrochloric acid. The yellow-coloured hydrochloric acid extract was evaporated to dryness and gave a dark-greenish hygroscopic powder. This residue was largely of an inorganic nature, and contained 39·8 per cent. of ash.

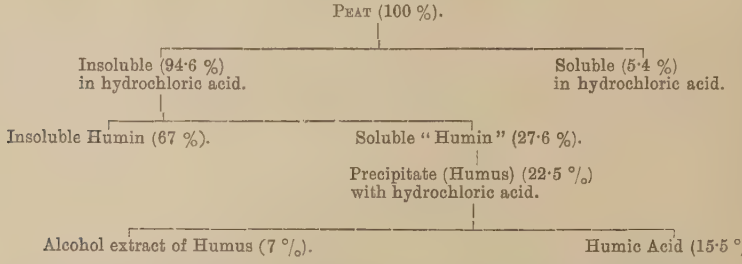
The peat, after treatment with dilute hydrochloric acid, was digested with excess of a 4 per cent. ammonium hydroxide solution, containing 20 grams of ammonium chloride per litre, and filtered. The insoluble portion was washed with dilute ammonia until the washings were colourless, and these were added to the filtrate.

At this stage difficulty was encountered owing to the colloidal nature of the insoluble humin. A Hele-Shaw stream-line filter was used to separate the colloid matter. The type employed was different from the usual model. It consisted of a hollow, grooved stem, perforated near the base and fixed on a circular porcelain stand. At the top of the stem was a screw-thread on which could be screwed a cone-shaped head, perforated through the apex. When in position this perforation coincided with the hollow centre of the stem.

The top part of the filter is unscrewed. On the perforated stem are placed a sufficient number of rings of filter-paper, to make a column extending from the base to about half-an-inch up the thread of the screw. The head is rescrewed. The top end of the filter is now attached by means of a rubber tube to a flask and thence to the vacuum pump. The filter is then wetted with

distilled water and placed in the liquid to be filtered. The liquid is drawn through into the filter flask, and the solid builds up a cake on the surface of the filter. This solid can be removed when desired by withdrawing the filter from the beaker and drawing air through it until dry, when the solid falls off easily and cleanly.

The insoluble portion is known as the insoluble humin, and contains the more resistant cellulosic constituents of the peat. It dried to a hard black dense mass resembling coal. The dark-coloured filtrate, when acidified with hydrochloric acid, gave a flocculent precipitate (humus), which was filtered off, again using the stream-line filter. The precipitate was repeatedly washed with dilute acid until the washings were colourless. The yellow-coloured filtrate contained ammonium chloride principally. The acid-washed precipitate was allowed to dry on the filter, when it broke up into large irregular lumps which could be easily removed. Repeated extraction with absolute alcohol separated this humus into two fractions.


Peat (100 %).
Insoluble (94·6 %) in hydrochloric acid.
Soluble (5·4 %) in hydrochloric acid.
Insoluble Humin (67 %).
Soluble "Humin" (27·6 %).
Precipitate (Humus) (22·5 %) with hydrochloric acid.
Alcohol extract of Humus (7 %).
Humic Acid (15·5 %).

The percentage weights of the fractions obtained from peat are given in Table II. Each of these fractions was then subjected to distillation under reduced pressure.

II.—*Comparative Distillation of Insoluble Humin and Humic Acid under Reduced Pressure.*

(1) *Distillation of Insoluble Humin.*—Insoluble humin (44 grams) was introduced into the copper distillation flask, which was fitted up as previously described. It was exhausted to a pressure of 9 mm. The bath was heated to 290° C. and the distillation flask immersed in it. A rapid evolution of gas took place, and the pressure rose to about 50 mm. Heating was discontinued for some time until the pressure was between 10 and 20 mm., and heating was so regulated that this range of pressure was maintained throughout the distillation. After an hour the stream of gas slackened considerably and the temperature of the bath was raised. At the end of four hours no more gas was being evolved. When the flask was immersed in the hot bath whitish vapours distilled over into the two receivers, and at the end of the experiment an aqueous solution containing a little tar was found to have collected in the cooled receiver, while the first mainly contained tar. A light powdery coke remained in the distillation flask. The percentage weights of the different fractions are given in Table I.

(2) *Distillation of Humic Acid.*—Humic Acid (14 grams) was introduced into the distillation flask, and the experiment carried out, as already described. Initially gas was given off much more rapidly in this case, and the pressure rose to 80 mm., though heating was discontinued shortly after immersion of

the distillation flask in the bath. At the end of an hour the stream of gas had slackened considerably, and after two hours had become almost negligible. The experiment was, however, continued for four hours. The percentage weights of the fractions are given in Table I, and also the percentage of coke, tar, and aqueous extract after separating the tar.

From these results will be seen the remarkable difference in the amount of tar obtained from the two substances. Humic acid gives only a trace of tar. The amount of coke and tar-water is much higher in the case of the humic acid, while the "gas and loss" is lower—probably indicating a more mild decomposition. The results obtained from a distillation of peat under similar conditions are appended for purposes of comparison. It will be seen that the insoluble humin resembles the peat much more than does the humic acid. In the case of peat, however, the amount of "gas and loss" is much higher than for either of the others.

III.—*Comparative Vacuum Distillations of Small Quantities of each of the Fractions for examination of the Gases Evolved.*

1 gram samples of each of the fractions were distilled under reduced pressure, and the gases evolved collected and examined, as in Part I.

(1) *Distillation of Insoluble Humin.*—Insoluble humin (1 gram) was introduced into the distillation flask, and the apparatus exhausted. As soon as the flask was plunged into the molten bath (temp. 290°) gas was evolved, and the pressure rose to 50 mm. Heating was discontinued until the pressure fell to a few millimetres, and was then resumed, regulating it so that no appreciable change of pressure occurred. 50 c.c. of gas collected in an hour, and after this the rate of evolution of the gas slowed down, until at the end of four hours it had ceased altogether. The percentages of the various liquid fractions agree with those already obtained by the authors in the larger distillation. The gas was collected in three fractions. The result of the analysis of the gas in the first two tubes is given in Table II. From these figures it can be seen that there is a considerable falling off in carbon dioxide during the experiment, accompanied by a corresponding increase in saturated gases and unsaturated hydrocarbons. Carbon monoxide showed a slight increase.

(2) *Distillation of Humic Acid.*—The gases evolved from 1 gram of humic acid, when subjected to vacuum distillation, were collected and examined. The experiment was carried out as already described. In this case gas was evolved much more rapidly in the beginning of the experiment, and very little tar distilled over. The percentages of the various fractions obtained from the 1-gram sample agree with those obtained in the previous larger-scale distillation of humic acid, as given in Table I. The gas was collected in three tubes, but the amount collected in the third was small, and was not analysed. The results of analysis of the gas, as given in Table II, show a decrease in carbon dioxide in the second fraction, though not so marked as in the case of the insoluble humin. There is a corresponding increase in the other gases, notably in the carbon monoxide content.

(3) *Distillation of the Hydrochloric Acid Extract of Peat.*—1 gram of the hydrochloric acid extract was next subjected to vacuum distillation, under the same conditions. Gas evolution was slow. 20 c.c. of gas collected in 25 mins., and after this the rate of evolution slackened considerably. While the distillation was in progress a violet colouration was noticed in the delivery tube from the distillation flask. The percentages of the various fractions

collected are given in Table I, and the result of the gas analysis is given in Table II.

(4) *Distillation of the Alcohol-soluble portion of Humus.*—1 gram of the alcohol-soluble portion of humus was distilled under reduced pressure, and the gas evolved collected and analysed. Gas was copiously evolved in the beginning of this experiment. After 30 mins. the rate of evolution of the gas slackened considerably. A violet colour appeared in the connecting tube between the two receivers. This resembled the colour already noticed during the distillation of the hydrochloric acid extract of peat, and did not appear during the distillation of any of the other fractions. The percentages of the different products of distillation are given in Table I, and the result of the gas analysis in Table II.

(5) *Distillation of Acetone Extract of Peat.*—Acetone extracted 10 per cent. of a wax-like material from peat, and 1 gram of this extract was distilled under conditions similar to those used for the other fractions. Gas was not evolved so vigorously in this instance. After the experiment, it was seen that a pure white, glistening wax had collected in the first receiver, while a dark, shiny, wax-like substance remained in the distillation flask, and nothing had collected in the cooled receiver. The percentages of the various fractions collected are given in the following table:—

Residue in the distillation flask	57 per cent.
Crystalline wax collected at 60° C.	26 per cent.
Fraction collected in cooled receiver ...	trace
Gas and loss	17 per cent.

The wax collected in the first receiver was examined, and found to be soluble in cold alcohol, acetone, ether, or petroleum ether, but considerably more soluble in the hot solvents. About one-third of the residue in the distillation flask was soluble in acetone. The results of the gas analysis are given in Table II.

TABLE I.

	Peat.	Insoluble Humin.	Humic Acid.	Hydrochloric Acid Extract.	Alcohol-soluble portion of Humus.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent
Residue (Coke), &c. ...	48·1	48·7	54·2	43	27
Distillate collected at 60° C.	14·6	12·4	1·2	1	49
Distillate collected in cooled receiver	14·9	21·3	29·6	42	14
Gas and loss	22·4	17·6	15·0	14	10
Tar	17·2	19·9	1·9	—	—
"Tar-water"	12·3	13·8	28·9	—	—

TABLE II.

	Insoluble Humin.			Humic Acid.			Hydrochloric Acid Extract.	Alcohol-soluble portion of Humus.	Acetone Extract of Peat.
Per cent. of Peat	67·0			15·5			5·4	7·0	10
Total volume at N. T. P. of Gas evolved from 1 gram. ...	93·8 c.c.			87·7 c.c.			28·5 c.c.	39·9 c.c.	26·0 c.c.
	Fraction I.	Fraction II.	Fraction III.	Fraction I.	Fraction II.	Fraction III.	—	—	—
Volume at N. T. P. ...	c.c. 43·7	c.c. 42·9	c.c. 7·2	c.c. 44·4	c.c. 42·2	c.c. 1·1	—	—	—
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Carbon Dioxide	73·1	6·0	—	83·3	62·8	—	62·7	76·2	71·4
Carbon Monoxide	14·0	18·8	—	8·0	24·9	—	1·2	11·4	2·4
Unsaturated Hydrocarbons	2·7	8·0	—	3·0	4·6	—	12·5	11·5	15·9
Residual Gases (saturated)	10·2	67·2	—	5·7	7·7	—	23·6	0·9	10·3

On examining the figures in Table I, it will be seen that the percentages of the various fractions (coke, tar, etc.) obtained from the insoluble humin approximate very closely to those obtained from peat. Humic acid and the hydrochloric acid extract give very little tar. Humic acid gives the highest percentage of coke, and the alcohol-soluble portion of humus the lowest.

An examination of the figures given in Table II for gas analysis shows that the difference in composition between the gases evolved from the various fractions is not so marked as the difference in volume. Humic acid and insoluble humin give the largest volumes. None of the fractions gives as great a volume of gas as peat, from 1 gram of which 112·2 c.c. of gas were obtained under the same conditions.

SUMMARY.

Peat, owing to the nature of its formation, consists of a large number of varying types of substances, mainly of an organic nature. Distillation experiments on such a material must, of necessity, give rise to complex mixtures. It is considered that, for the purpose of simplifying the type of distillation product, the most promising line of attack would be to first fractionate the peat into its various constituents, and then investigate these separately. The method employed in obtaining the various fractions from peat is described. These fractions were subjected to rapid distillation under reduced pressure, and the products of distillation collected in two receivers—one kept at a constant temperature of 60° C., and the other immersed in a freezing mixture. Coke.

distillate collected at 60° C., distillate collected at 0° C., and gas were the crude products in each case, but the percentage of these varied considerably for the different fractions. Humic acid and the hydrochloric acid extract gave very little tar. The highest percentage of "coke" was obtained from the humic acid, and the lowest from the alcoholic extract of humus.

The gases evolved when each of the fractions was vacuum-distilled were collected and analysed. The difference in composition of the gases was not so marked as the difference in volume. The greatest volume of gas was obtained from the insoluble humin.

A modified arrangement of plant for the distillation of peat under mild conditions is now being investigated, and it is intended to proceed with this work before an attempt is made to interpret further the above results.

No. 32.

THE CORRELATION OF NUTRITIVE VALUE WITH DRY MATTER CONTENT OF PASTURES.

By E. J. SHEEHY, F.R.C.Sc.I., B.Sc., M.R.I.A. (Department of Lands and Agriculture).

[Read February 22. Published separately April 7, 1927.]

The quantity of green material produced per unit area is, within limits, an indication of the nutritive value of a pasture. Coarse herbage, with an abundance of miscellaneous plants, renders certain pastures obviously inferior, but in the case of those which are readily eaten by stock, the weight of green fodder produced per annum is one of the most important, if not the most important, of the factors controlling the stock-carrying capacity of a given area. The total annual weight of herbage cannot be judged solely by the height to which the plants grow in the ungrazed pasture in a given period. On the "crag,"[1] for instance, the grass is quite short, while in the much less fertile neighbouring pastures it is apparently more abundant, because of the taller habit of growth. Thickness and density of growth, though a less obvious feature, is, perhaps, a more potent factor in determining total produce than the rate of growth of the herbage. In view, however, of the remarkable difference in the stock-carrying capacity of pastures, all of which apparently produce an ample growth of grass, there is a strong presumption in favour of a qualitative difference, no less important, perhaps, than the quantitative one.

A considerable amount of investigation has been made into the quality of pastures designated poor and rich, according to their capability to maintain and fatten stock, but, so far, the nutritive difference has not been definitely explained by examination, either of the herbage, or the soil on which it grows.

Literature.

Wilson (1), referring to the comparative values of pastures for sheep, incidentally draws attention to the importance of a high percentage of dry matter in grasses, and gives figures to show that the dry matter content of clovers in a pasture is less than that of grasses. Aitken (2) analysed the air-dried herbage sent him by a number of farmers from rich, mediocre, and poor pasture fields, and seeks to show a difference in chemical content which may be correlated with the difference in the quality of the pasture. In view, however, of the fact that the figures represent the analysis of one set of samples

[1] "Crag" is a local term applied to a type of pasture occurring in the West of Ireland on very thin soil overlying a limestone rock rich in phosphates.

taken from paled-in plots, they may not represent the average chemical composition of the well-grazed pasture for the entire year: and, in any case, the chemical differences determined between poor and rich pastures were small, and not always constant. Very much reliance cannot be placed on the interpretation of these analyses in view of Wilson's (3) results, which show the necessity, in working out comparative figures, for analysing the herbage at different stages of growth. In connection with Aitken's investigation, the soil and subsoil were also analysed; and evidence was brought forward to show that the richer pasture soil contains more of the chief fertilising constituents, especially of phosphates, both total and available. Armstrong (4), after making an analysis of a large series of pastures, concludes that "the herbage of the best grazing land may be twice as rich in nitrogen and phosphate as that of a poor pasture," and that "the large difference appears to be directly determined chiefly by the proportion of wild white clover present, and, indirectly, by the percentage of available phosphates in the soil," and that "the choicest grazing land is invariably associated with soil rich in available phosphates." Hall and Russell (5) made an investigation into the causes of the high nutritive value of certain fields in Romney Marsh, and, while they found a difference in the habit of growth between poor and rich pastures—namely, a stemmy growth in the one and a leafy growth in the other, the floral type being constant—the differences revealed by the ordinary methods of chemical analysis were very small. They conclude that the ordinary method of analysis of herbage is inadequate to detect differences in feeding value. Though the soils of the fattening fields possessed no constant outstanding properties, the high rate of nitrogen assimilation and the relatively large proportion of total phosphoric acid are noteworthy. Fagan and Jones (6) made successive analyses throughout the growing season of a number of individual grasses from special plots, and showed that the leaf contains a higher percentage of protein and a lower percentage of fibre than the stem, and that the ratio of true to crude protein is higher in the leaf than in the stem. In view of these findings, they drew special attention to the proportion of leaf to stem in a well-grazed pasture.

Experimental.

In view of the great variety of pastures found in Ireland, it was decided to initiate an investigation, to be afterwards conducted on a wider scale, into the significance of the differences in their nutritive value and the explanation thereof. The extremely fertile grass lands of Meath and the medium pastures on the average tillage soil of Kildare were chosen for comparison. Both are healthy pastures, providing an abundance of herbage, and are sufficiently near one another to preclude differences resulting from climate. The Meath pasture is extremely nutritive, and able to support the maximum number of stock; the Kildare one, on the other hand, is, as regards the stock-carrying and fattening qualities, not more than one-third, or one-half, as valuable as the other. The field chosen for investigation in Meath is situated at Peterstown, near Trim, and belongs to Mr. Kennedy, who kindly gave permission to have removed whatever samples of grass and soil were desired. The field has been in grass for a very long period of years, and the appearance of the surface suggests that it was very indifferently laid out. In Kildare, Mr. Speirs kindly gave permission, and much assistance, to secure samples from one of his fields at Burtown. The pasture was of a semi-permanent nature, and was recently laid out to grass, and well seeded.

In order that the two pastures might be kept under continuous observation, it was decided to transport representative plots, and to transplant them, side by side, at the Albert College, Glasnevin. A plot of each pasture, one yard square, was selected, and the turf, to a depth of 9 inches, removed, as far as possible, unbroken. The underlying soil was then taken out in successive horizontal layers of 8, 5, 6, and 8 inches respectively, which were kept separate from one another. Suitable receptacles were prepared at Glasnevin by excavating the soil to a depth of 3 feet, and building on the subsoil a retaining wall 3 feet high, enclosing a ground space 3 feet square. The concrete receptacles were lined inside with puddle, which rendered them impervious to liquids; the top of the wall was built flush with the surrounding soil. The bottom of the receptacle was unlined, and consisted of the subsoil *in situ*. The various layers of soil and subsoil taken from each plot were deposited in the appropriate receptacle in the reverse order to their removal, so that the entire cubic yard of material was placed in its new situation as if it had been removed *en bloc*. The deep subsoil of the transported soil was placed directly in contact with the Glasnevin subsoil, so that free movement of liquid upwards and downwards was allowed, while lateral movement from the surrounding soil into the experimental plot, or *vice versa*, was prevented. Four such chambers, each one cubic yard in content, were filled. In each of two plots, containing samples from Kildare and Meath, respectively, the old sward was placed grass upwards, so that it continued to grow as if it had not been disturbed. These were labelled plots 1 and 2. In the other two, again containing samples from Kildare and Meath, respectively, the top 9-inches of sod was inverted, and the red surface sown with the following grass-seed mixture, each plot being liberally seeded, and receiving exactly the same quantity of seed:—

Perennial rye grass	8½ grams per plot.
Italian „ „	3½ „ „ „
Meadow fescue	3 „ „ „
Timothy	1½ „ „ „
Cocksfoot	1½ „ „ „
Alsike clover	1 „ „ „
White „	1 „ „ „

These were labelled plots 3 and 4. The transportation of all the samples, and the seeding of plots 3 and 4, took place in February, 1922. Plots 1 and 2 allowed the herbage on the old pastures, and plots 3 and 4 the herbage on freshly laid-down pastures from Kildare and Meath, to be examined under identical conditions and circumstances.

The chemical composition of the herbage was determined in the usual manner. The botanical examination was kindly undertaken by Mr. Gorman, of the College of Science. Digestibility experiments were performed on guinea pigs, rather than on larger animals, because of the small supply of herbage available. The animals were housed in chambers so constructed that the urine and fæces were collected separately, and the same animals were used for all the digestion experiments conducted in each year. The experimental animals were put on a diet consisting solely of grass for some days, then a "marker" was fed, so as to colour the fæces; the experimental grass was then supplied for some days; a " marker " was again fed, and then ordinary grass diet continued. The fæces vacuated between the two " markers " was taken as the undigestible portion of the experimental grass consumed.

The general appearance and botanical composition of plots 1 and 2, *i.e.*, the old pastures, in June, 1922, and again in June, 1923, was as follows:—

	Meath.	Kildare.
General appearance (1922):	Moderately tall, brilliant green herbage, containing little or no "weeds."	Short, "weedy" herbage: dense sole due to narrow-leaved grasses among the "weeds."
Botanical Composition (1922):	Perennial rye grass (Principal Grasses.) Crested dogstail (Principal Grasses.) Meadow foxtail (Principal Grasses.) R. S. meadow grass (Principal Grasses.) S. S. " " (Principal Grasses.) Fiorin (Principal Grasses.) Yorkshire fog Red fescue Cocksfoot Golden oat grass White clover Sorrel (In very small quantities.) Bulbous buttercup (In very small quantities.) Mouseear chickweed (In very small quantities.)	Red fescue Crested dogstail Perennial rye grass Cocksfoot Yorkshire fog Quacking grass White clover Red clover Birdsfoot trefoil Field woodrush Plantain Catsear (Abundant.) Daisy (Abundant.) Hawkbit (Abundant.) Knapweed (Abundant.) Yarrow (Abundant.) Bulbous buttercup (Abundant.) Creeping buttercup (Abundant.) Cowslip (Abundant.) Mouseear chickweed (Abundant.) Ladysbed straw (Abundant.) Ox-eyed daisy (Abundant.)

	Meath.	Kildare.
General appearance (1923):	Moderately tall, brilliant green herbage; little or no weeds; perennial rye grass the predominant plant.	Short, stunted herbage; very "weedy"; thick sole; narrow-leaved grasses plentiful; the perennial rye grass and fescue stunted and narrow-leaved.
Botanical Composition (1923):	Perennial rye grass (bulk of herbage) Yorkshire fog Fiorin Red fescue Cocksfoot Smooth-stalked meadow grass Crested dogstail Meadow foxtail White clover Sorrel Buttercup	Perennial rye grass (stunted) Red fescue Crested dogstail Yorkshire fog Cocksfoot Sweet vernal Birdsfoot trefoil Red clover Field woodrush Catsear Knapweed Narrow-leaved plantain Buttercup Yarrow Cowslip Daisy

The proximity of the plots enabled the dry matter content of the herbage to be determined under identically similar conditions. The comparable plots were cut to exactly the same degree of bareness, the cuttings being made at the same time, and always on the afternoon of a dry day succeeding a dry night, so that no surface water adhered to the outside of the leaves and stems. The

samples were weighed immediately after cutting, and dried in a steam-oven. The following table shows a number of results from cuttings made at different times from plots 1 and 2, *i.e.*, the old pastures:—

Date of Cutting.	Percentage Dry Matter.	
	MEATH.	KILDARE.
June 14, 1922	27·4	20·8
Sept. 15, „	27·2	20·2
June 16, 1923	27·6	23·6
July 21, „	29·3	19·2
Oct. 20, „	27·0	22·9

The chemical composition of the dry matter of two composite samples, each representing a number of cuttings taken respectively from plots 1 and 2, *i.e.*, the old pastures, during the year 1922, is as follows:—

	MEATH.	KILDARE.
	Per cent.	Per cent.
Protein (crude)	12·5	14·1
Fat and Oil (ether extract)	3·03	3·1
Fibre	21·5	20·75
Ash	7·6	9·4
Nitrogen-free extract	55·37	52·65

The plots provided luxuriant grazing at the time of each cutting, but the pasture was, by intermittent cuttings, prevented from approaching the hay stage. Comparison of the figures for protein and fibre with those recently published in connection with a very extensive piece of work by Woodman, Blunt, and Stewart (7), on the nutritive value of pasture, shows that the above figures are intermediate between the protein and fibre content of hay and of very closely grazed pasture.

Two digestion experiments were carried out on plots 1 and 2, in 1922, and one was conducted in 1923, and the results are presented in the following table:—

	EXP. 1 (1922).		EXP. 2 (1922).		EXP. 3 (1923).	
	MEATH.	KILDARE.	MEATH.	KILDARE.	MEATH.	KILDARE.
	(Grams.)	(Grams.)	(Grams.)	(Grams.)	(Grams.)	(Grams.)
Total dry matter consumed as grass	144·9	110·0	131·6	98·0	240·0	230·0
Total dry matter in fæces ...	53·0	39·0	43·5	30·2	96·0	87·0
Total dry matter digested ...	91·9	71·0	88·1	67·8	144·0	143·0
Percentage of dry matter digested	63·4	64·6	66·9	69·1	60·0	62·2

Plots 3 and 4, which were laid down with similar seed mixtures in the Spring of 1922, showed a considerable difference throughout their growth. The Meath plot grew more vigorous, stronger, and greener plants than the Kildare one, and the leaves of the plants were broader, and more luxurious. The botanical composition of plots 3 and 4 in June, 1923, and June, 1924, was as follows:—

	Meath.		Kildare.	
Botanical Composition (1923):	Italian rye grass Perennial rye grass Cocksfoot	Greater proportion of Italian than perennial.	Perennial Italian Cocksfoot	Greater proportion of perennial than Italian.
	Cranesbill Buttercup Silver weed	One plant each.	Soft brome (few plants)	
Botanical Composition (1924):	Perennial rye grass Cocksfoot Italian rye grass Yorkshire fog Golden oat grass S. M. meadow grass R. S. „ „ Red fescue Meadow fescue Silver weed Buttercup.		Perennial rye grass (Principal Grass) Cocksfoot (small quantity) Italian rye grass Yorkshire fog Plantain Daisy	few plants.

It is very interesting to note the change in the character of the herbage in both plots. The only determinations made on these two plots were those of dry matter content, which worked out as follows:—

Date of Cutting.	Percentage of Dry Matter.	
	Meath.	Kildare.
June 7, 1923	29·6	30·6
July 13, „	28·6	27·0
Oct. 20, „	31·3	29·1

A repetition of the experiment was made in 1926, when, however, determinations were made of the dry matter only. A sward from a rich pasture from the Albert College Farm, Glasnevin, was included, together with the Meath and Kildare pastures. All three were transported, and, as before, placed on their own subsoils to a depth of 3 feet in the special receptacles already used, and which were located within a few feet of one another, in a field on the Albert Farm, Glasnevin. The plots were laid down in January, 1926, and during the growing season the herbage was frequently clipped, or, rather, plucked, so as to prevent flower formation, while maintaining an abundant mat of pasture. The dry matter content of the herbage on each of the three plots was determined on three occasions throughout the growing season, the samples being taken on each occasion from all plots at the same time, when the herbage was perfectly dry.

Duplicate plots, which were allowed to grow into flower, were used, in conjunction with the continuously cropped ones, to determine the botanical

composition, which was made in May, and was kindly undertaken by Mr. M. J. Gorman, of the College of Science. The botanical composition, in May, 1926, was as follows:—

MEATH.	KILDARE.	GLASNEVIN.
Perennial rye grass } Principal	Perennial rye grass	Perennial rye grass
Fiorin } grasses.	Crested dogstail	Cocksfoot
Meadow foxtail	Sweet vernal	Fiorin
Rough-stalked meadow grass	Cocksfoot	Yorkshire fog
Cocksfoot	Downy oat grass	Rough-stalked meadow grass
Red fescue	Red fescue	Crested dogstail
Yorkshire fog	White clover (a little)	White clover
Timothy	Perennial red clover.	Daisy
White clover (fairly plentiful)	Field woodrush	Plantain
Crowfoot, creeping and bulbous	Birdsfoot trefoil	Dandelion
Mouseear chickweed	Ladysbed straw	Crowfoot
	Plantain	Mouseear chickweed
	Daisy	
	Catsear	
	Knapweed	
	Cowslip	
	Creeping crowfoot	
	Yarrow	

The Meath herbage constituted a dense grassy pasture, showing a fair proportion of white clover, but practically no weeds. The Kildare one, on the other hand, was a very mixed pasture, containing no constituents in outstanding proportions, but possessing a large number of rosette plants and weeds. The Glasnevin pasture was somewhat intermediate, but approached more closely to the Meath type, being a predominantly grassy pasture, though the herbage was not so vigorous as that of the Meath plot.

The dry matter content, as determined by drying to constant weight in a steam-oven, was as follows:—

Date of Sampling.	MEATH.	KILDARE.	GLASNEVIN.
	Per cent.	Per cent.	Per cent.
End of June	29·8	22·3	27·0
„ „ August	30·2	22·6	25·3
Early October	37·0	29·5	36·3

Discussion.

The amount of data collected in this investigation, especially in so far as chemical composition is concerned, is meagre; but the findings in respect of chemical analysis of dry matter agree with those of previous investigators, in that the figures, as determined by the ordinary routine analysis, cannot be correlated with nutritive value. There is a disparity between the two pastures in the results for nitrogen content, and some difference in ash; but, curiously, the balance is in each case in favour of the Kildare sample. The superiority of the Meath pasture is associated with its wonderful fattening qualities, with which the chief constituents concerned are fats and carbohydrates. Proteins may also be used for fattening, and they possess, for that purpose, about the same value as carbohydrates. Protein is, however, principally concerned with muscle production, while ash functions in the construction of bone as well as

in the ordinary processes of metabolism; but for these purposes Kildare pastures have not been shown to be less suited than those of Meath. The possibility of the superiority of one pasture over another being partly explained by a greater nitrogen content is not, however, precluded; and, in connection with ash, Dr. Orr (8) and his collaborators at the Rowett Institute of Nutrition have demonstrated that the relative amounts of certain mineral constituents determine the relative nutritive value of certain pastures which they have examined. Apparently, however, the Kildare pasture is as efficient as the Meath one for the purpose of building up an animal's body, and it would appear unlikely that qualitative difference in mineral content could be correlated with their relative nutritive value for fattening stock. Further experimental work on these pastures will, however, include qualitative mineral analysis. Difference in vitamin content may be excluded as a possible factor contributing towards difference in nutritive value, because all green leaves possess these materials in sufficient quantities for ordinary requirements.

The results of the digestibility experiments go to show that there is little or no difference in the utilisation of the two pastures by guinea pigs. In fact, the Kildare herbage showed consistently, though slightly, higher results. It is possible, however, that the utilisation of pasture by cattle and sheep is different from that obtained by the use of guinea pigs.

When the figures for dry matter content of the old pastures are compared, a remarkable difference in favour of the Meath grass is shown. A definite weight of the Meath herbage contains about 25 per cent. more dry matter than the same weight of Kildare herbage; so that a beast may absorb about 25 per cent. more nutriment from the Meath grass than from the same weight of Kildare pasture. It may, however, be contended that this factor could operate only when restricted quantities of grass are fed, and that it would not have the same effect under conditions of unlimited freedom on well-clothed pasture. But an animal's capacity for assimilation is limited by the size of its alimentary tube, and working on the premise that two similar bullocks, one on the Meath and the other on the Kildare pasture, eat to the capacity of their stomachs, then obviously the former assimilates about 25 per cent. more nutriment than the other.

If the relative nutritive value of the two pastures under investigation may be correlated with their dry matter content, and if a difference in the percentage of dry matter determines, at least partly, the nutritive difference in this case, what is the explanation of the phenomenon, and how far may it be extended to other pastures? While the Meath pasture consists almost entirely of a close sward of grasses and clovers, the Kildare herbage includes, in addition, a variety of broad-leaved miscellaneous plants. When the different constituents of the Kildare sample are grouped and the water content determined, the following results are obtained:—

	Grass.	Clover.	Miscellaneous Plants.
Percentage of Dry Matter	26·3	25·8	17·0

The grass portion of the Kildare herbage shows a percentage of dry matter which approximates closely to that of the total Meath herbage, which is predominantly grass. Examination of the results for dry matter content of plots

3 and 4—*i.e.*, the Meath and Kildare newly laid-down pastures, in which, on account of the abundant seeding, few or no miscellaneous plants had appeared into the second year—shows very little difference between them in respect of dry matter. It appears, therefore, a fair inference that the difference in dry matter content between the old Kildare and Meath pastures is not due to a difference in this respect between the grass portion of their herbage, but rather to the varying proportion of miscellaneous plants, and perhaps clovers, contained therein. Possibly, varying proportions of dry matter in the grasses, even in grasses of the same type from different pastures, may partly explain differences in their nutritive value, but in the case of the two pastures under review, the outstanding feature is the correlation between the dry matter of the total herbage and the relative proportions of weeds and clovers. The prime Meath pasture is constituted of a very dense mat of medium-sized grass leaves packed closely together, and interlaid with slender grass leaves and an admixture of small clovers. Much of the Kildare pasture is, on the other hand, covered with broad-leaved miscellaneous plants, covering space which would produce very much more dry matter were the herbage a close matting of grass leaves.

A confirmation of the view that the percentage of dry matter affords a partial explanation of the difference in nutritive value between pastures was forthcoming from an examination of samples of herbage from Limerick pastures, kindly forwarded in sealed bottles by Mr. Gleeson, Itinerant Instructor in Agriculture. A sample taken, from what he described as prime pasture, consisted of about 97 per cent. of medium and narrow-leaved grasses, with a little white clover, and no miscellaneous plants—almost exactly the kind of sample collected from prime Meath pastures. A fairly good Limerick pasture was constituted as follows :—

Grass	77·8	per cent., containing	28·4	per cent, of dry matter.			
Clover	8·0	„	„	19·2	„	„	„
Miscellaneous plants	14·2	„	„	19·6	„	„	„

And a fair pasture from the Department's farm at Athenry was made up as follows :—

Grass	55·9	per cent., containing	29·2	per cent. of dry matter.			
Clover	11·8	„	„	19·3	„	„	„
Miscellaneous plants...	32·2	„	„	15·5	„	„	„

The grass leaves contain, approximately, the same high proportion of dry matter in all the pastures examined; while the "weeds," and, to a lesser extent, the clovers, contain a relatively low weight of dry matter.

The results of the 1926 test confirm the earlier findings, namely, that the dry matter content of the Meath pasture dealt with was considerably greater than that of the herbage from the Kildare pasture. In this connection, it is interesting to note that the dry matter content of the Glasnevin pasture is intermediate between the others, though approaching closely that of the Meath plot, a result which may be correlated with the appearance and constitution of the pasture as described above, as well as with its comparative stock-carrying capacity or nutritive value.

SUMMARY.

1. There is a very marked difference in botanical character between the Kildare and Meath pastures examined. In addition to the grasses, the former contains a large proportion of miscellaneous plants, many of them broad-leaved, some birdsfoot trefoil, and red and white clover; while the Meath pasture consists of densely packed grass leaves, with an admixture of clover.

2. The chemical composition of the dry matter of both samples differs so little that they may be regarded as similar in this respect. This knowledge was obtained from a limited amount of data, which did not include determinations of the various minerals.

3. The digestibility of both pastures, as tested on the guinea pig, a typical herbivor, is practically similar.

4. The percentage of dry matter in the Meath pasture is considerably greater than that in the Kildare one, there being a difference of about 25 per cent. This constitutes one definite factor of difference between the two pastures.

5. Meath herbage is, therefore, about 25 per cent. more nutritive than an equal weight of Kildare herbage, because of the greater proportion of dry matter.

6. The dry matter content of the two pastures may be correlated with the relative proportions of miscellaneous plants, and, to a lesser extent, of clovers present; the clover containing a lower percentage of dry matter than the grass, and the miscellaneous plants much less dry matter than either.

7. The relative nutritive capacity of other pastures examined may be correlated with the relative proportions of grass, clover, and miscellaneous plants, which determine the dry matter content.

8. The dry matter content is put forward as one factor in the partial explanation of the nutritive differences between certain pastures.

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No. 33.

THE MAINTENANCE REQUIREMENTS OF CATTLE ON DIFFERENT RATIONS AND AT DIFFERENT RATES OF PRODUCTION; WITH A NOTE ON "DYNAMIC ACTION."

By JAMES WILSON, M.A., B.Sc.

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In two earlier papers ("The Variations in the Quantity of Food required by Cattle for Maintenance and Production," Scient. Proc. R.D.S., 1925, **18**, Nos. 7 and 10), it was shown that the gross food consumed and the portion required for maintenance vary with the nature of the ration and the rate at which the animal is producing. In the first, it was taken for granted that maintenance rises directly with the rate of production; but, in the second, it was indicated that the rise is accelerated as the rate of production rises. The main purpose of this paper is to confirm the indication given in the second paper.

Maintenance has usually a restricted meaning, namely, the food, or food energy (gross or metabolisable), required to drive the organism, keep the body warm, and manipulate the food, when the animal is neither gaining nor losing fat or flesh. In this paper, it means the metabolisable energy, whether drawn from the body or digested from the food, or both, required to drive the organism, keep the body warm, and digest the food, at any rate of production: from complete starvation to the fullest possible diet.

Naturally, maintenance is at a minimum during starvation; for, then, no food needs to be manipulated. When food is fed, however, additional organs are set to work, and those working before are working harder. The food has to be seized, masticated, carried to the stomach, raised to the temperature of the body, tossed about, disintegrated, and digested. It must then be forced through the intestines, the digested portion absorbed, and the undigested expelled. If not already in forms usable by the animal, the digested food must be converted into such forms, and, if not to be used at once, carried to, and stored in appropriate places. Obviously, maintenance must rise, and rise seriously, with the intractability and quantity of the food, and with the rate of increase.

Maintenance may be found by the Kellner or the Armsby method. Kellner had his bullocks confined in a Pettenkofer chamber, and fed on rations of different kinds and sizes. The energy in the whole ration, in the dung and marsh gas—that is, in the undigested portion—and in the urea was determined. By subtracting the energy in the dung and marsh gas from that in the whole ration, the digestible energy is found; and, by subtracting from that the energy in the urea, the metabolisable energy in the ration is found. The nitrogen and carbon retained by the bullock were determined, thence the fat and flesh, and thence the energy; and, by subtracting that from the metabolisable energy in the ration, the energy required for maintenance is calculated.

Armsby had his bullocks confined in a calorimeter, and fed on rations of different kinds and sizes. The total, and the metabolisable, energy in the

ration, and the heat emitted by the bullock—that is, the energy required for maintenance—were determined; and, by subtracting the heat emitted from the metabolisable energy, the energy retained by the bullock is calculated.

Thus, by the Kellner method, the energy retained by the bullock is determined directly, maintenance calculated; by the Armsby method, maintenance is determined directly, the energy retained by the bullock calculated.

The following table contains Kellner's and Armsby's essential data, expressed in convenient forms. The food consumed is expressed in lbs. of long fodder and concentrates to the live hundred-weight. In many of Kellner's experiments, dried beet slices were fed, and these, because they behave as concentrates, are reckoned as concentrates. The gross Calories in the rations and those required for maintenance are converted from what they were, as actually observed or calculated, to what they should have been had the animals been all the same weight, 9 cwts., *i.e.*, 1,008 lbs., which is near the standard commonly adopted, 1,000 lbs.

It may seem a super-refinement to give the Calories down to units and decimal places, but the figures are Kellner's and Armsby's.

KELLNER'S EXPERIMENTS.

Bullock.	Period.	Dry matter consumed per live cwt.		Proportion of long fodder.	Gross energy in ration.	Energy used for maintenance.	Energy retained by bullock.	Proportion of gross energy retained.
		in long fodder. (Meadow hay).	in concentrates.					
		lbs.	lbs.	Per cent.	Cals.	Cals.	Cals.	Per cent.
II ...	—	1·49	—	100	34490	14521	830·9	2·3
XX ...	—	1·41	—	100	34190	15158	957·9	2·8
V ...	—	1·45	—	100	32280	12874	1487·6	4·6
VI ...	—	1·34	—	100	31440	13219	1683·3	5·3
A ...	—	1·31	—	100	30160	15879	1548·8	5
VI ...	2b	1·3	·27	83	37820	15696	4062	10·7
V ...	2a	1·4	·30	82	38260	15198	4268·5	10·1
V ...	2b	1·36	·29	82	38060	14024	4014·2	10·5
VI ...	3	1·26	·45	74	42170	17287	5413·8	12·8
H ...	7	1·06	·53	67	41620	18316	5762·9	13·9
J ...	2	1·21	·62	66	42200	19288	5205·8	12·3
H ...	2	1·15	·9	66	41830	17623	6258·3	15
F ...	1	1·25	·7	64	42590	17874	4592·6	10·8
G ...	3	1·14	·66	63	40890	18027	3938·3	9·6
E ...	3	·91	·57	62	41290	18594	5923·1	14·3
E ...	4	·89	·66	57	42610	20959	3145·9	7·4
E ...	2	·96	·72	57	41810	18983	4336·8	10·4
E ...	1	·98	·79	56	41790	18812	5917·7	14·1
D ...	1	·97	·79	55	43030	17760	6616·6	15·4
D ...	3	·9	·82	52	48330	20095	8931·0	18·5
D ...	4	·87	·88	50	49400	21151	8221·9	16·6
D ...	2	·93	1	48	48510	19038	8769·5	18·1

Bullock.	Period.	Dry matter consumed per live cwt.		Proportion of long fodder.	Gross energy in ration.	Energy used for maintenance.	Energy retained by bullock.	Proportion of gross energy retained.
		in long fodder.	in concentrates.					
		(Meadow hay).						
		lbs.	lbs.	Per cent.	Cals.	Cals.	Cals.	Per cent.
C ...	1	·87	1·16	43	44740	18201	7477·9	16·7
B ...	4	·73	1·04	41	42370	20964	5846·2	13·8
C ...	3	·82	1·31	39	51850	21484	9748·5	18·8
C ...	2	·82	1·34	38	50040	19457	8886·4	17·8
B ...	1	·81	1·45	36	51710	23657	8338·8	16·1
B ...	3	·74	1·32	36	50270	23557	8758·1	17·4
B ...	2	·77	1·38	36	51160	22002	8314·8	16·2
H ...	4	·58	·58	50	28050	14159	2057·1	7·4
J ...	4	·61	·61	50	28460	14606	2301·5	8·1
G ...	3	·62	·66	49	28800	14609	1777·3	6·2
F ...	3	·65	·69	49	29610	14763	2058·7	7
F ...	5	·60	·77	44	36300	16153	4487·5	12·3
G ...	5	·58	·77	43	35210	15814	4672·1	13·3
J ...	3	·61	·90	40	35090	16532	4995·8	14·2
J ...	6	·58	·87	40	34590	15639	4561·2	13·2
H ...	6	·55	·83	40	34050	15160	6378·8	18·7
F ...	4	·63	·97	39	35710	15945	5388	15·5
G ...	4	·61	·94	39	35290	16374	5367·2	15·2
H ...	3	·57	·92	38	35640	16811	5808·7	16·3
H ...	5	·56	1	37	38120	16877	7873	20·1
F ...	6	·54	·95	36	34980	16578	4627·7	13·2
J ...	5	·59	1·04	36	38700	17790	7139·7	18·5
		(Hay and straw).						
IV ...	2	1·35	·29	82	38030	15890	1761·6	4·6
III ...	2	1·33	·29	82	38030	15495	3006·7	7·9
IV ...	3	1·38	·4	78	41850	17427	4010·1	9·6
III ...	3	1·35	·4	77	41860	17118	4076·7	9·7
III ...	4	1·23	·46	73	44010	18358	5287·3	11·5
H ...	1	1·19	·60	66	42290	17868	2736·7	6·5
J ...	1	1·24	·63	66	42590	17893	3565·8	8·3
F ...	2	1·26	·66	66	43770	18005	4229·6	9·7
G ...	1	1·24	·67	65	42790	17840	3683·4	8·6
ARMSBY'S EXPERIMENTS.		(Mixed hay).						
D ...	2/211	·45	·46	50	16200	9779	– 230	—
D ...	3/211	·92	·93	50	30974	14053	7283	23·2
G ...	2/211	·25	·48	34	10957	8641	– 1358	—
G ...	3/211	·67	1·31	34	32279	13821	5480	17

Bullock.	Period.	Dry matter consumed per live cwt. in long fodder.	Dry matter consumed per live cwt. in concentrates.	Proportion of long fodder.	Gross energy in ration.	Energy used for maintenance.	Energy retained by bullock.	Proportion of gross energy retained.
		(Red clover hay). lbs.	lbs.	Per cent.	Cals.	Cals.	Cals.	Per cent.
I	3/179	·70	·17	80	16625	10240	– 2230	—
I	4/179	·67	·72	48	28089	14040	3560	19·5
		(Timothy hay).						
B	1/200	·91	·45	67	18419	8782	– 449	—
B	1/207	·83	·42	66	20160	9905	– 84	—
A	1/200	·72	·50	59	21193	9558	1397	6·5
A	1/207	·66	·45	59	22969	9995	1890	8·2
B	2/200	·79	·69	53	23050	10266	1179	5·1
B	2/207	·80	·77	51	26420	11128	2489	9·5
A	2/200	·70	1·11	39	32435	13039	4467	13·7
A	2/207	·63	1·02	38	35218	13676	6518	18·5
		(Alfalfa hay).						
J	1/–	1·61	·66	72	38030	15783	5237	13·8
J	3/–	·97	·40	71	23320	11049	1095	4·7
J	2/–	·72	·30	71	16330	9668	– 746	—
J	4/–	·49	·20	71	10870	8245	– 2398	—
F	3/209	·19	·39	33	8200	6882	– 1992	—
F	2/209	·3	·63	32	13510	7955	– 145	—
F	1/209	·54	1·13	32	22510	9811	2266	10
		(Red clover hay).						
I	1/179	·92	—	100	19140	10536	– 2971	—
I	2/179	·69	—	100	13560	9881	– 3849	—
I	1a/186	·58	—	100	12570	10115	– 4675	—
I	2a/186	·96	—	100	21410	10688	– 578	—
I	3a/186	·8	—	100	17650	10087	– 1990	—
		(Alfalfa hay).						
F	6/209	·85	—	100	10600	7311	– 2899	—
J	7/–	·93	—	100	15420	8451	– 1413	—
H	5/212	·99	—	100	14130	7754	– 884	—
F	5/209	1·30	—	100	16580	8362	– 1140	—
H	3/212	1·68	—	100	24250	10323	807	3·3
J	6/–	1·70	—	100	27940	11231	1000	3·1
H	1/212	2·13	—	100	30440	11745	2203	7·2
F	4/209	2·15	—	100	28760	11838	318	1·1
J	5/–	2·19	—	100	34170	13139	2282	6·7

Bullock.	Period.	Dry matter consumed per live cwt. in long fodder. (Timothy hay).	Dry matter consumed per live cwt. in concentrates.	Proportion of long fodder.	Gross energy in ration.	Energy used for maintenance.	Energy retained by bullock.	Proportion of gross energy retained.
		lbs.	lbs.	Per cent.	Cals.	Cals.	Cals.	Per cent.
A ...	3/207	·66	—	100	13110	7629	– 1545	—
B ...	3/207	·73	—	100	12840	8066	– 2238	—
A ...	3/200	·74	—	100	12270	7600	– 2468	—
B ...	3/200	·93	—	100	13660	7465	– 2142	—
A ...	4/207	1·07	—	100	21610	9279	656	3
A ...	4/200	1·22	—	100	22640	8396	– 223	—
B ...	4/207	1·35	—	100	21660	9803	– 32	—
B ...	4/200	1·38	—	100	22500	8028	– 368	—
		(Mixed hay).						
D ...	5/211	·46	—	100	7940	8219	– 4744	—
D ...	4/211	·86	—	100	15360	9314	– 2344	—
D ...	1/211	1·52	—	100	27298	11710	– 362	—
G ...	1/211	1·75	—	100	27110	9329	293	1·1

The data do not grade well, and are arranged but roughly in the table. As they are arranged, however, it may be seen that maintenance varies with

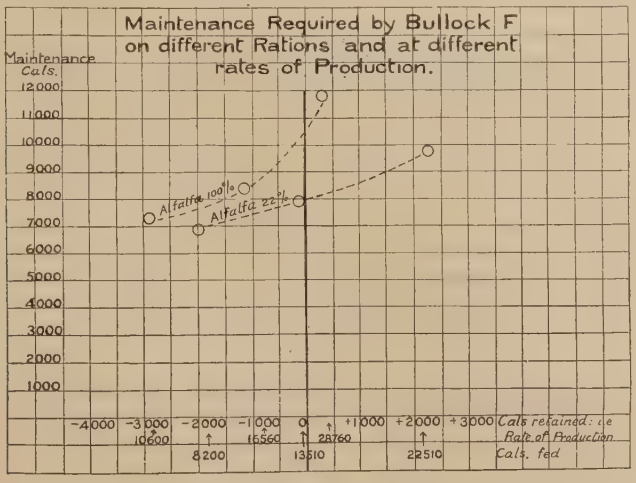

FIG. 1.

the kind and quantity of long fodder when long fodder alone is fed, with the proportion of long fodder to concentrates when both are fed, and with the size of the ration and the rate of production. It may also be seen that a greater proportion of the energy in the ration is retained by the animal as more food

is fed and the proportion of concentrates to long fodder is increased. These and other inferences may be made clearer and more definite if the data are expressed diagrammatically.

In fig. 1, the Calories required for maintenance are plotted against those retained by Armsby's bullock *F*, when fed with alfalfa hay alone, and with a ration containing 22 per cent. of alfalfa hay and 78 per cent. of concentrates. From this diagram it is obvious that, at equal rates of loss or gain, more food has to be consumed and more metabolisable energy is necessary for maintenance with hay alone than with hay and concentrates. It is also obvious that maintenance rises with the food consumed and with the rate of increase. It

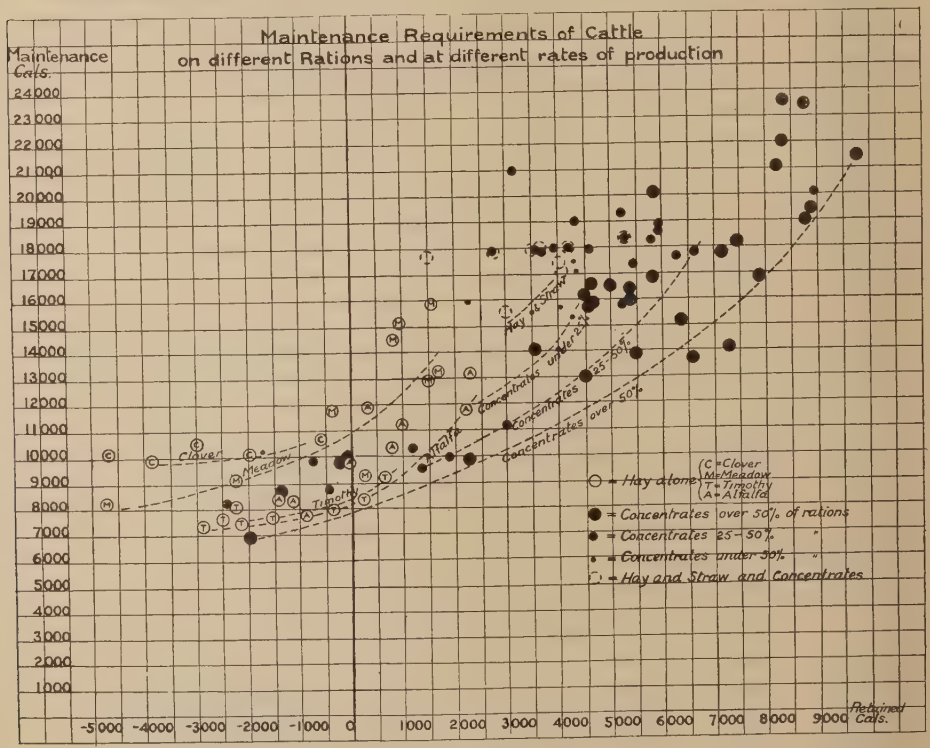

Fig. 2.

does not rise directly, however, but is accelerated. But, with which is it accelerated? With food, or rate of increase, or with both? According to the diagram, it is accelerated with both; but, though the acceleration with food or rate of increase cannot be determined separately, it is obvious from the diagram that increase in long fodder is the chief cause of acceleration. The upper line in the diagram indicates that, if more and more long fodder be fed, a stage may be reached when more energy will be required for maintenance than can be extracted from the food by the animal. If it could consume enough hay, the bullock might starve, just as Zuntz's horse starved on wheat straw.

In fig. 2, the whole of Kellner's and Armsby's data are plotted, as in fig. 1: maintenance against rate of production, and, by rough implication,

against quantity of food. The lines in the diagram are drawn through what are approximately the lower margins of the plotting marks for each kind of ration, that is, through the plotting marks for the rations which, for one reason or another, were working most efficiently, and are, therefore, most fairly comparable. Horses cannot be compared for speed unless equally fed, trained, and ridden; rations cannot be compared for efficiency unless under equal opportunities. The chief inferences to be drawn are that—

1. When fed alone, alfalfa and Timothy hays are most efficient—require least for maintenance—then meadow hay, then, unexpectedly, red clover hay.
2. Rations containing concentrates are more efficient than rations containing hay alone, at all rates of production.
3. Rations containing hay and concentrates are more efficient than rations containing hay and straw—therefore, than rations containing straw—and concentrates.
4. Rations rise in efficiency the more concentrates and the less long fodder they contain.

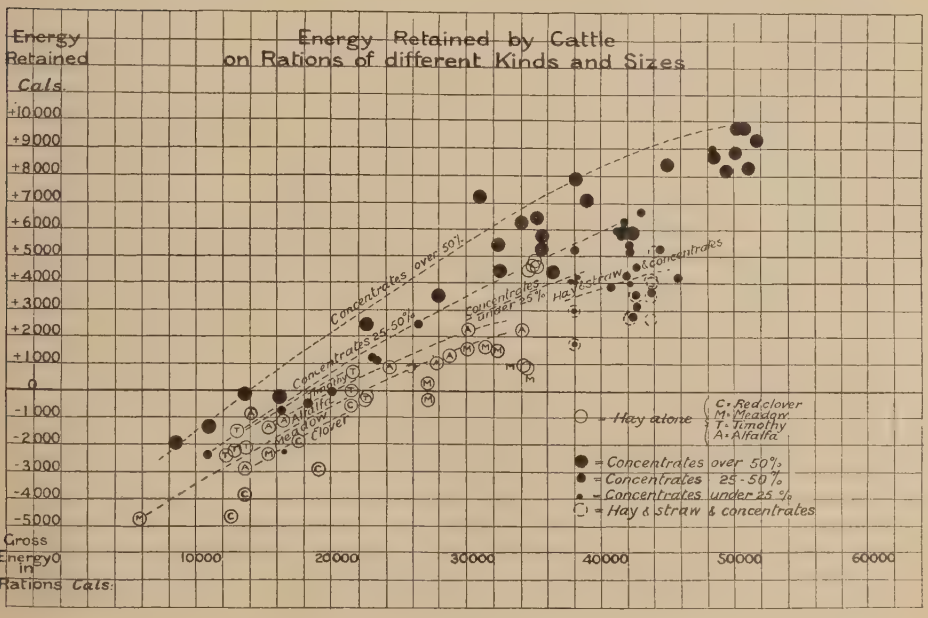

FIG. 3.

Fig. 3 is merely the economic obverse of the second, and shows that the greater the proportion of concentrates the more the economy; that long fodder and concentrates are more economical than long fodder alone; and that long fodder alone is incapable of rapid production—the first essential to profits in stock-feeding, as in any other industry.

The data connected with Kellner's experiments are to be found in his "Untersuchungen über Stoff- und Energie-Umsatz des erwachsenen Rindes bei Erhaltungs- und Produktionsfutter," 1900. The data connected with Armsby's experiments are to be found in Armsby and Fries's "Net Energy Values of Feeding Stuffs for Cattle," Armsby and Fries's "Net Energy Values for Alfalfa Hay and for Starch," and Forbes and Kriss's "Revised Net Energy Values of Feeding Stuffs for Cattle," all published in the Journal for Agricultural Research, in 1914–15, 1918, and 1925.

Note on "Dynamic Action."

The finding in this paper has a bearing on what, since Rubner so named it, human physiologists call "dynamic action." It is observed that, after a rapidly digestible meal, especially one containing much protein, the consumer's heat emission rises. Denying that any energy is required for digestion—"there is no 'work of digestion'"—the physiologist has to find some explanation of this increased heat emission, and suggests that, when the food comes in contact with the substances already in the body, some kind of interaction takes place, and heat is emitted. But, though it may seem unduly spontaneous, is this interaction anything else than digestion—than digestion which must be paid for in energy?

Though it must require less energy than is required for the food consumed by bullocks, is it safe to assume that the food consumed by man and by the animals with which the physiologist experiments requires no energy at all for its manipulation? Manipulation may be divided into three main sections: disintegration and digestion, conversion, and storing. Little energy may be required for the disintegration and digestion of human foods; but are the carbohydrates to be converted into usable substances, the proteins to be split into amino-acids, and the amino-acids built up again into proteins, with no expenditure of energy? Lusk refers to experiments with diabetics: "It was known that glycocoll and alanin were completely convertible into glucose in the diabetic organism, whereas glutamic acid was in part so converted, three of its five carbon atoms passing into glucose, the other two being oxidised . . . Glycocoll and alanin produced powerful effects, lasting eight and five hours respectively, whereas on giving those quantities of glucose into which the amino-acids are convertible only an almost negligible influence is observable."[1] That is to say: substances which have to be converted bring about heat emission; substances which have not to be converted do not.

And may there not be a further explanation of part, at least, of this so-called "dynamic action"? The storing capacity of the human body—especially of the fat human body—is small; the storing capacity of the adult human body for protein is very small. May it not be that, when a large, readily digestible, meal has been consumed, the stuffs digested and converted, being more than can be stored, must be eliminated, and can be eliminated only with the production of heat? And, if the meal contain much protein, may not the heat production be excessive?

[1] Science of Nutrition, Third Edition, 1921, p. 240.

No. 34.

THE PRODUCTION OF THE RESTING-SPORES OF *PHYTOPHTHORA INFESTANS* ON POTATO TUBERS.

By PAUL A. MURPHY, Sc.D., M.R.I.A.

(Plate XVIII.)

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The question of the resting-spores of *Phytophthora infestans*, so long a vexed one, has lately dropped into the background, and most authorities would have been ready to say that the oospores are not known to occur in nature. Long searches, carried out by many investigators in the past, had failed to reveal them on any part of the potato itself, but the work of Jones, Giddings, and Lutman (5), Clinton (1), and Pethybridge and Murphy (9) showed that the fungus was capable of producing more or less perfect sexual organs and oospores on certain artificial media. There was still, however, no proof that these occurred on the natural host, and the first reference to their being found there (apart from some earlier work, which is referred to in the discussion at the end of this paper) was made by Pethybridge (8) at the International Potato Conference, in London, in 1921, where photographs were also exhibited of sexual organs and spores as produced on potato tubers, but not in pure culture. The present author was solely responsible for that work, and it is shortly described below. In 1922 Miss Löhnis (6) recorded the development of somewhat malformed oogonia, some of which contained spores, in cultures on portions of potato tubers *in vitro*. This was confirmed by Miss de Bruyn (2), who, further, found apparently perfect oogonia containing parthenogenetic spores on portions of potato, and in cultures on bog soil (3), both *in vitro*. In the latter paper this author also showed that perfect sexual organs and spores were freely produced in pure cultures on cereal straw.

The present paper records the finding of sexual organs on potato tubers under a variety of conditions. As first discovered, they were present in impure cultures on naturally infected tubers; they were then found repeatedly in pure cultures; and, finally, in the soil surrounding potato tubers, under conditions which represent those of nature. These organs are usually abortive oogonia, but sometimes they contain spores, and occasionally antheridia are present as well, resulting in apparently perfectly formed sexual spores, for the antheridia appear to have functioned. This is the first record of the perfect sexual organs on potatoes, and of the production of resting-spores on tubers under natural conditions in the soil, which indicates, in the author's opinion, the natural place of formation in nature.

First Discovery of Sexual Organs on Tuber Fragments.

The resting-spores were first found on two separate fragments of a naturally infected tuber in test tubes in May, 1921. Nine of these tubes had been prepared in the previous March, and on the date mentioned these were opened and

examined, prior to discarding them. The cultures were not pure, there being a *Penicillium* sp. present in one case, and a *Macrosporium* sp. in another, as well as bacteria.

The potato fragments in question were found to be considerably decayed, and they were covered with fungous growth, consisting of masses of old conidiophores and dead conidia of *P. infestans*. There were also present among this mass a large number (upwards of 90 in one field of view) of yellowish to hyaline rounded bodies of a large size, and provided with a tapering neck, recalling oogonia. These structures occurred singly, or two, three, or more, together at the ends of the hyphae. The wall was distinctly double-layered down as far as the base of the neck. The latter was exactly of the tapering shape found on oogonia provided with amphigynous (7) antheridia, through which the neck passes, but no antheridia could be found in these cultures. Most of the oogonia were empty, or contained disorganised contents; but some included spherical spores, with a smooth wall, which varied in thickness (fig. 1). The contents of the undifferentiated oogonia did not appear to be in a living condition, but most of the spores were perfect in all respects, so far as could be judged, being filled completely with coarse protoplasm. The spore wall consisted of two slightly differentiated layers—the outer, yellowish-hyaline, and much the thinner; the inner, greyish-brown. The thickness varied from about 1μ to 4·5μ in the best specimens.

These structures at once recalled the imperfect oogonia secured in pure culture by Clinton (1), Jones, Giddings, and Lutman (5), and Pethybridge and Murphy (9). The oogonia as developed in pure culture are so peculiar, and the resemblance between them and the bodies under discussion was so striking, that there was no doubt the two were identical. They agreed well in size also, for the transverse diameters of twelve oogonia produced on the potato varied from 31 to 43μ (average, 36μ); and six more or less perfect spores measured from 25 to 30μ in diameter (average, 27μ); while the size of these organs in pure culture averages, according to Clinton, 38–42μ and 24–35μ, respectively; and, according to Pethybridge and Murphy, 38μ and 30μ, respectively. Except for the apparent absence of antheridia, the organs were quite as well formed as those produced on artificial media, such as oat agar. This applies in particular to the parthenogenetic spores (fig. 1), although some of the less perfect specimens were thin-walled, and small in size.

Sexual Organs produced in pure Culture on Potato Portions.

The same bodies were subsequently found on a number of occasions in pure cultures on fragments of potato tuber *in vitro*, as the following experiments show. In the first of these a large number of portions of naturally infected tubers were transferred to sterile test tubes containing moist cotton wool, with the usual precautions against sepsis, of which eight developed pure cultures of *P. infestans*. These were examined at intervals during the next two months, with the following results:—Oogonia appeared (in some cases within 17 days) among the aerial mycelium in four tubes, and were abundant in one; but practically all were abortive, and failed to produce spores. Two perfect sets of organs, consisting of oogonium, amphigynous antheridium, and oospore (figs. 7 and 8), were, however, found in one tube. No sexual organs were formed within the potato tissue. Some of the same lot of blighted tubers began to produce similar oogonia when cut open and kept for twelve days in a closed dish, while still in the full tide of conidium production, and it was established that the oogonia sprang from the same mycelium as did the conidiophores.

In another experiment, three plugs of a healthy live potato tuber were similarly placed in test tubes, incubated for four days, found sterile, and then inoculated with a pure culture of *P. infestans*. The fungus grew in two of the cultures, but one became contaminated with bacteria. The other developed no sexual organs during the first two months, but when again examined six months later, when it was still pure, abortive oogonia were present.

A third experiment was as follows:—Portions of a naturally infected tuber were removed aseptically, and incubated under microscopic control on set plates of potato agar for seven days. No contamination appearing, they were then transferred to sterile test tubes containing moist cotton wool. Good growth was made, which consisted entirely of *P. infestans*. No sexual organs appeared within the first two months, but when examined again after seven months oogonia were found in all three tubes—sometimes close to the medium, and sometimes below, or in, the cotton wool, 2 cm. away, where fresh conidia were being produced. The cultures were still pure.

Live potato plugs are not an ideal medium, because it is difficult to prove the entire absence of other organisms, at least in the early stages. To overcome this possible objection, another experiment was conducted. Three portions of healthy live potato tuber were placed aseptically in sterile test tubes on moist cotton wool, and incubated for 21 days. At that time, when they were apparently sterile, smears were taken from their surfaces and transferred to oat agar slants. Two of the latter remained sterile, and the other developed a bacterial growth. The two plugs which were proved to be sterile were then inoculated with a pure culture of *P. infestans*. Growth in these cases is usually very slow, the fungus finding it difficult, apparently, to penetrate into the potato, but eventually it grew vigorously in one tube, and when examined after about five months, a typical spherical oogonium, containing an oospore, was found in the cotton wool below the potato (fig. 2). Conidiophores and conidia of *P. infestans* were still being produced. The other tube yielded no sexual organs. Both cultures were still pure.

These experiments prove beyond question that *P. infestans* forms its sexual organs on the surface of potato tubers among the aerial mycelium. The oogonia were found to be comparatively common, and occasionally they contained a resting-spore, which appeared to be perfectly formed, but antheridia were practically absent. The organs were indistinguishable from those produced on artificial media[1] in pure culture, although the proportion of perfect ones was smaller, and they exactly resembled the bodies produced on fractions of potato tubers in impure culture, which have already been described. The oogonia were found to arise on somewhat narrow branches of thicker hyphae growing out from the potato, so far as their connection could be traced. The only exception to this rule is shown in fig. 14, in which an apparent oogonium, of narrow, elongate shape, and with undifferentiated contents, was found attached to the germ-tube of a conidium on the surface of a tuber. The shape of the oogonia was spherical to sub-spherical, or more usually elongate club-shape, with a long, gradually tapering neck, at the base of which a plug or septum was often present. The walls of the oogonia were distinctly double-layered, as a rule, down to the neck, and their colour was yellowish to hyaline. In some cases, parthenogenetic spores were evidently in process of formation, for the contents were contracted into an oosphere; in other cases, apparently perfect

[1] The brown material which surrounds the oogonia produced on oat agar, which is a result of the action of an exudate from the oogonium on the medium, is absent in the cultures on potato tubers.

smooth oospores were present, with walls up to 3·5μ thick, which were evidently parthenogenetic in origin (figs. 10 and 11), but usually the contents were disorganised, and no spore was present (figs. 5, 6, 12, and 13). In two cases in pure culture on potato the oogonia were provided with amphigynous (7) antheridia (figs. 7 and 8), which evidently had functioned, since they were empty of contents when found, and well formed spores were present. The description of these organs is as follows:—Oogonium, yellowish to slightly brown, except stalk, which is hyaline; antheridium, hyaline, and empty; spore wall colourless, not visibly layered, smooth, 2 to 3μ thick; spores, 24 to 26μ in diameter; contents, fairly finely granular, almost homogeneous, lighter in centre.

Formation of Sexual Organs on Tubers in the Soil.

That the sexual organs may occur in nature, and that there also they are found among the mycelium on the surface of the potato tuber, will now be shown.

A whole tuber and two half tubers were artificially inoculated with a pure culture of *P. infestans*, and planted near the surface of three pots of sterilised soil. Three weeks later the tubers were examined *in situ*, when two of them showed nothing of interest, but one of the cut pieces exhibited a strong growth of the blight fungus. This was breaking out in pustules at the lenticels on the uninjured skin, and was spreading appreciably in the soil from the edge of the cut surface, which was downwards, an abundance of conidia being present, but no sexual organs.

When the seed-piece was lifted out of its bed, a faint greyish mycelium was present on the floor, which, on examination, was found to consist almost entirely of *P. infestans*, together with a little *Rhizoctonia*. The conidiophores and conidia of the former were present, and almost every fraction of the non-septate mycelium was forming oogonia, which were full of protoplasm, and looked quite normal, except that no antheridia were present. This was one of the most abundant productions of sexual organs which has ever been seen, but, as stated below, none of them developed further after the tuber was removed. Some, however, were already producing spores at that time, one of which is shown in fig. 9. Examination of the seed-piece (which was attacked by bacterial rot on portion of the cut surface) showed that the oogonia were confined to the soil, and that none were present in the tissues.

When the mycelium in the soil was again examined two days later, it was found that the contents of practically all the oogonia were contracted and granular, and no further growth had been made. It was evident that they had been drawing on the tuber for nutriment, and when the connection was broken they ceased to develop. The tuber was then replaced in the soil, but no further development took place, for it rotted badly.

Discussion.

The work which has just been described clears up three points, namely:—(1) that the perfect sexual organs and resting-spores of *P. infestans* are produced on the natural host; (2) that, so far as the potato is concerned, they are formed in nature on the surface of the tubers, which simplifies the task of determining their practical importance; and (3) that the sexual organs, as produced on the potato plant, are similar to those produced on artificial media, in that they both tend to be imperfect.

The last point has a bearing on previous work on this question, which will be referred to very briefly. The bodies found by Smith (10) in potato leaves have no resemblance to those now described; and if any more evidence were

needed that they did not belong to *P. infestans* it is now available. Much the same may be said of Eriksson's (4) work. The bodies found by Smorawski (11) on aerial mycelium springing from the surface of a naturally infected tuber are rather more like what we have found, and he was probably dealing with incipient oogonia, although the bodies at the side can hardly have been antheridia. Gambell's case was probably similar; for, as reported by Jones *et al.* (5), he found on the surface of potato tubers carrying a pure culture of *P. infestans* thick-walled smooth bodies, 22 to 23μ in diameter, "possibly immature resting-spores," but the manner in which they were formed could not be determined. The position in which these bodies were found in both cases is significant.

The only questions still to be settled are: how commonly do these bodies occur, how frequently they form perfect resting-spores, and to what extent the last function as originators of blight in subsequent years. Future work will have to determine this, but the present writer's opinion is that the resting-spores are not of very great importance. In the first place, they are not, apparently, of very general occurrence, for, though they have been found on blighted material from three different sources, they cannot be said to be common. On the other hand, they occur more frequently than previous work would indicate, the reason for their being overlooked so long being, probably, that they are produced outside of the host and not within its tissues. Again, taken as a whole, the organs are more imperfect than those occurring on some artificial media, antheridia are apparently all but absent, and well-formed resting-spores are rare. Nevertheless, once it is known that such resting-spores do occur in nature, they cannot be entirely left out of account when considering the sources of blight infection on the foliage, for it is not impossible that further work may discover more abundant and more perfect specimens.

Summary.

Following the discovery of oogonia of *Phytophthora infestans,* some of which were abortive, and some of which contained parthenogenetic resting-spores, on portions of potato tubers in impure culture *in vitro,* further experiments were instituted, which showed that similar bodies were sometimes produced in pure cultures on portions of potato tubers, and that very occasionally a perfect set of organs, consisting of oogonium, antheridium, and oospore, was produced. Oogonia and parthenogenetic spores were subsequently found on the surface of a cut tuber in the soil, which is considered the natural place of formation.

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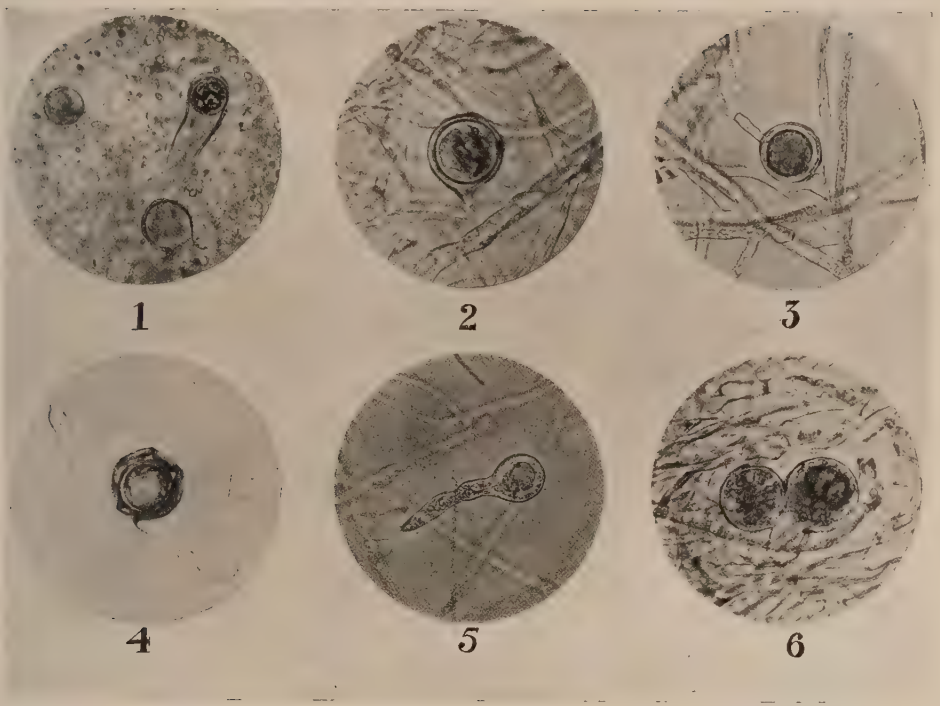
DESCRIPTION OF PLATE.

Fig.

1. Oogonia of *Phytophthora infestans*, some containing parthenogenetic spores, as first found on potato tubers in impure culture. (× 240.)
2 and 3. Oogonia of *P. infestans* with parthenogenetic spores, produced in pure culture on potato tuber. (× 350.)
4. Oogonium and oospore produced in pure culture on potato tuber. On dissection, an amphigynous antheridium was found to be present, as shown in fig. 8. (× 350.)
5 and 6. Usual types of abortive oogonia, produced on potato tubers. (× 350.)
7 and 8. Two perfect sets of organs, consisting of oogonium, amphigynous antheridium, and oospore, produced in pure culture on potato tubers *in vitro*. Same organs are shown in figs. 4 and 8. (× 350.)
9. Oogonium and parthenogenetic spore, produced on surface of tuber under natural conditions in soil. (× 350.)
10 and 11. Parthenogenetic spores, produced in pure culture on potato tubers. (× 350.)
12 and 13. Usual types of abortive oogonia, produced on potato tubers. (× 350.)
14. Apparent case of oogonium developing on germ tube of conidium on surface of potato tuber. (× 350.)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No. 35.

SOME FURTHER CASES OF THE PRODUCTION OF DISEASED SHOOTS BY POTATO TUBERS ATTACKED BY *PHYTOPHTHORA INFESTANS*, AND A DEMONSTRATION OF ALTERNATIVE SOURCES OF FOLIAGE AND TUBER INFECTION.

By PAUL A. MURPHY, Sc.D.,
AND
ROBERT M'KAY, A.R.C.Sc.I.

(Plate XIX.)

[Read January 25. Published separately June 1, 1927.]

Although the potato blight fungus, *Phytophthora infestans*, reappears unfailingly every year on the aerial portion of the potato plant, the exact method by which it first makes its way from the tubers in which it hibernates to the foliage still remains a somewhat open question.[1] It was established by de Bary (6) that the sprouts produced by infected tubers may be invaded by the fungus, and yet may survive long enough to reach the surface of the ground, where conidia are produced, which become centres for the further spread of the disease. Yet this, the only proved method by which the disease arises, has been observed by four investigators only since de Bary's time—namely, Jensen (11), Pethybridge (21), Melhus (15, 16), and, lastly, Salmon and Ware (23), by whom the work of the other authors mentioned has been summarised.

The present paper records, in the first place, as shortly as may be, still another case of blight appearing for the first time on the surface of shoots from two naturally infected tubers, which just managed to come above the ground under open field conditions in 1926. This is the first time that this has been seen in the open by the present authors; but in experiments under laboratory conditions with artificially infected tubers in 1922 the same phenomenon was observed more than once, and was, indeed, reproduced practically at will. In connection with the latter experiments, which are shortly described, attention is particularly drawn to the fact that the soil immediately surrounding a diseased tuber usually becomes highly contaminated with mycelium and conidia, whether diseased sprouts reach the surface or not. The infection of tubers of the new crop, either direct from the parent plant or from this source, is also demonstrated.

It is now known with some degree of probability that the annual reappearance of blight on potato foliage is not exclusively associated with the production of diseased shoots which reach the surface of the ground, and the question is discussed as to how far the observations recorded above provide an alternative source of infection. In this connection, the bearing of our own

[1] Other possible methods of hibernation are discussed later.

experiments on the possibility of hibernation in the soil is also considered; and, finally, the production under natural conditions of sexual organs—generally only oogonia which are mainly abortive, but which may contain spores—is referred to, these bodies being fully described elsewhere (18).

The Production of Infected Aerial Shoots by Diseased Tubers in the Field in 1926.

Earlier experiments, some carried out in Canada and some in Ireland, in which naturally infected tubers were planted in the open, sometimes in considerable numbers, had invariably given negative results; for such plants as were produced remained healthy about as long as neighbouring plants derived from sound tubers. The successful experiment was carried out as follows:—

On May 20th, 1926, sixteen naturally, but not severely, blighted tubers, which carried some normal-looking sprouts, were planted in two shallow boxes with partially open bottoms, containing fresh loam from a permanent pasture mixed with a little leaf-mould, with which they were covered to the depth of about an inch. These materials had never previously been used for potatoes, and no manure was added. The boxes were then sunk in the ground in an open space in the middle of a large field of corn. The plants came up well, and nothing unusual was noticed until June 25th, when some of the smaller shoots of two plants, one in each box, were found to be dead. The boxes were then taken in, and the plants were carefully dug up and examined. One of the affected plants consisted of six stalks, of which two, which reached just a little above the soil level, were brown, moist-looking, and dead from base to tip. On examination, conidiophores and conidia of *P. infestans* were found on their surfaces. The remaining four shoots had brown, watery, discoloured spots just above the surface of the ground, on which the same fungus was present, but below this the shoots were healthy. Similarly, one or two isolated spots on the leaves yielded the same fungus after incubation.

The other affected plant was very similar—for three of its shoots, from 2 to 4 inches in length, were brown, and dead from their points of junction with the tuber practically to the tips, and the fructifications of *P. infestans* were readily found here again. Some of the remaining shoots of this plant, and of two of its neighbours, were slightly attacked in the same way as before, just above ground level, and the disease present was again proved to be blight. All the remaining plants were healthy in all their organs.

The experiment had not been visited for some little time before June 25th, and it was then evident that blight must have been present for a few days. It was clear, however, how the disease had originated on the five shoots of the two plants already described, which were killed, and which exactly resembled the shoots the invasion of which was watched step by step in the laboratory, as described below—namely, by spreading into them from the tuber direct. Furthermore, no other conclusion is possible, but that it had spread to some of the other shoots, just above the level of the soil, and to the leaves, by means of the conidia which were present on the dead shoots. There were no other potatoes near from which the blight could have come, and careful search failed to reveal any of the disease on the nearest crop (consisting of many early and late varieties), either then or throughout July.

The remarkable success of this experiment, in contrast with all the failures which had preceded it, is attributed to the fact that the potatoes were planted late, and were not deeply covered. The weather during the 37 days which the experiment lasted was as follows:—Mean max. temp., 62·8° F.; mean min. temp., 46·2° F.; total rainfall, 2·99 inches, falling on 22 out of the 37 days.

Production of Infected Aerial Shoots by Diseased Tubers in the Laboratory.

It is easy to demonstrate the fact that under laboratory conditions blighted tubers frequently produce diseased sprouts, some of which may be capable of penetrating through several inches of soil, and of carrying the parasite to the surface. The thinner the covering of soil, the greater is the chance of the diseased sprouts coming through. This has been observed several times; and, indeed, when the work was in progress in 1922, a special experiment was set up for the purpose of demonstrating the fact to the public at the Spring Agricultural Show of the Royal Dublin Society, which worked quite satisfactorily, the disease appearing on a dying shoot, which penetrated through three inches of soil, and appeared above ground. It is not proposed to go into the question in any detail, however, because it is felt that the applicability of such results to normal conditions out-of-doors is somewhat questionable.

The experiments were conducted in small, loosely-covered boxes, about 6 by 5 by 6 inches deep, having one removable glass side, through which the development of many of the shoots and roots could be observed (figs. 1 and 2). The tubers were artificially inoculated, and were planted with the sprouts close to the glass, covered in all cases by 3 inches of sterilised soil. Diseased shoots were usually produced, the behaviour of which was observed, but they did not penetrate through the three inches of soil unless certain conditions were provided. Our work in this respect was based on that of Melhus (15, 16), which was substantiated. The most favourable conditions for the emergence of diseased shoots were found to be obtained by placing the boxes in an incubator at a temperature of about 25° C., in the presence of sufficient moisture. In about two weeks, if the experiment was successful, *P. infestans* had reached the surface of the soil in diseased shoots, which died, and disappeared very quickly afterwards. This experiment was successfully carried out on two occasions in the spring of 1922. At lower temperatures, the growth of the sprouts was slower, and, although some of the shoots generally became diseased, they never emerged. On the other hand, the parasite almost invariably grew out into the soil to a certain extent, and this, the main object of these experiments, is dealt with in the next section.

Development of Phytophthora infestans in the Soil around Diseased Tubers.

The experiments now to be described deal with the growth of the fungus from diseased tubers, "sets," or sprouts, into the surrounding soil; and the possibility of this material serving as a source of aerial infection is discussed.

The experiments in question, and some of our other work dealing with the persistence of *Phytophthora infestans* in the soil (17, 19, 20), were performed before the appearance of the paper of Miss de Bruyn (7), which showed that this fungus was capable under certain conditions of living saprophytically for long periods, at least, in sterilised soil *in vitro*. This interesting and unexpected result led us, at first, to believe that we had missed the full significance of our own work, but further consideration and experience have again convinced us that the thesis originally propounded in 1922 by one of us (17) is correct—namely, that the fungus is capable of surviving in soil for an appreciable time, but one which, under ordinary conditions, is strictly limited.

The fact that the fungus could spread into the soil in this way was first realised in 1921, when fractions of a tuber, which had been inoculated from a pure culture a few days previously, were sterilised superficially, and buried 2·5 cm. deep in sterilised soil in small, loosely-covered beakers. On scraping away the soil after the lapse of 21 days in layers approximately 1·7 mm. thick,

perfect conidia of *P. infestans* were found in the soil to a minimum height of 1·33 cm. above the potato, as well as below it, and this soil was proved to be pathogenic to potato slices, from which the original fungus was recovered. The experiment was fully controlled, and there is no doubt that the infection was not present in the soil to begin with, but that it spread into it from the potato.

The spread of the fungus from whole and cut tubers planted in soil was then examined in the years 1921 and 1922. The boxes with glass panels, already described, were principally used. The tubers were artificially inoculated, incubated for a few days, and then disinfected superficially, after which the wounds were sealed with paraffin. The soil used, which was moderately moist, was sterilised, and proved to be free from blight infection. Three principal experiments were conducted in the laboratory during the winter at a somewhat low temperature—one with a whole tuber, and two with half tubers, with a control on each. The result was exactly the same in all cases. Some of the sprouts were invaded direct from each of the three tubers (a process which was observed directly), and in each case there was a considerable development of mycelium and conidiophores of *P. infestans* (and other fungi) in the soil in the immediate vicinity of the diseased sprouts and tubers, where numberless conidia were produced, by means of which blight was conveyed to potato tubers, from which *P. infestans* was recovered. The invasion of a sprout and the development of the blight fungus in the soil are illustrated in fig. 1. Of a total of 11 sprouts produced in the three experiments, 7 were invaded, and these were in all cases killed[1] before they emerged, and the fungus never reached the surface either within them or by growing independently through the three inches of overlying soil. The greatest extension through the soil was between 1 and 2 cm. above and below the tuber, and the soil beyond this was proved in all the experiments to be free from the fungus. This is in close agreement with the results of Jones, Giddings, and Lutman (12), and Melhus (16). Other fungi, radiating, in all probability, from the centre of disease, grew up visibly to the surface, but not the blight fungus.

The details of one typical experiment are as follows:—

14/12/'21.—Tuber inoculated 1·5 cm. from group of sprouts; incubated. *21/12/'21.*—Tuber dipped in spirit; wound sealed with paraffin; tuber planted whole in sterilised soil in box with glass panel, with sprouts next glass, and 7·5 cm. below surface of soil. Wounded healthy control tuber in similar box. Soil of both boxes tested and found non-infective on potatoes. *8th day after planting.*—One short sprout turning watery brown at junction with tuber. *10th day.*—Fungus present on base of discoloured sprout. *13th day.*—Fungus growth on sprout increasing and spreading up to 5 cm. in soil. *19th day.*—Disease, which gradually advanced up sprout, has now killed it. A fungus growth has appeared on surface of soil, but this is not *P. infestans*. *29th day.*—Glass panel opened. Fungus around dead sprout, but not that widely radiating in soil, proved to be *P. infestans*; numerous conidia in adjacent soil, some of which had germinated, some having produced zoospores, some germ-tubes, and some secondary conidia; ungerminated ones readily functioned as zoosporangia in water; a few were dead. Soil from just below and just above tuber produced blight in potato tuber, from which *P. infestans* was later recovered, but soil lying 3·5 cm. and 7·5 cm. above tuber failed to do so. *58th day.*—No trace of fungus in soil; tuber dug up. Sprout originally attacked had disappeared. Two adjacent ones were attacked at base and their growth stopped. Two perfect sprouts came up from other end of tuber. Soil from all levels no longer produced blight. *Control.*—No dead sprouts or fungus growth at any time. Roots more vigorous and better provided with root hairs. Soil from all levels, when tested on 29th day, produced no blight. *Result.*—Three sprouts of blighted tuber invaded, but their growth was stopped very quickly; fungus spread into soil, but never penetrated through sprouts or soil further than about 2 cm. from tuber.

[1] Injury is not confined to the sprouts, for, as may be seen from figs. 1 and 2, root development is seriously interfered with.

Generally similar results were secured in later laboratory experiments in 1922 with half-tubers artificially inoculated, and planted in sterilised soil in flower-pots. When cut tubers were used, there was sometimes a very copious development of the fungus from the cut surface, and viable conidia were found as late as 57 days after planting in the adjacent soil, by means of which blight was conveyed to healthy tubers, from which *P. infestans* was recovered. The fungus also emerged, however, through lenticels in the unbroken skin. It is concluded that the length of time the fungus survives in the soil depends on the permanence of the substratum on which it is being nourished.

The idea is general that the growth of *P. infestans* into the sprouts or through the soil demands very abundant moisture. This is not necessary, according to our experience, for the production of mycelium in the soil, although the boxes and pots used were kept covered. It has also been stated that the fungus easily succumbs in competition with other organisms; but we have found it in the soil in the company of numerous other fungi and bacteria, such as develop on sets suffering from blight followed by soft rot. In fact, the blight fungus seems to be able to hold its own under these conditions with its competitors, and to adapt itself to very different conditions. Thus, it lived for five months on the surface of a blighted tuber fragment, that was kept permanently covered with water, growing out vigorously into the liquid, and reaching the surface about 3 cm. above, where conidia were produced.

These experiments indicate that the production of diseased sprouts, which reach the surface of the soil, and produce conidia there, demands special conditions, as Melhus (15, 16) first pointed out, while the production of conidia below ground is, according to our experience, so common as to appear to be the rule. Although the problem of how these conidia, when produced subterraneously, can reach the surface has not been solved, the fact of their general occurrence cannot be left out of account. Brefeld (1, p. 116) believed, on theoretical grounds, that the fungus could grow through the soil, reach the surface, and produce blight on the foliage, but the maximum penetration through the soil in our experiments did not exceed a few centimetres; and, unless the tubers happen to be near the level of the ground, an independent agent would appear to be necessary to bring the fungus to the surface. Of such an occurrence, however, no proof is available, in spite of a variety of experiments on the subject which we have conducted, but it appears reasonable to us that the operations of weeding, hoeing, and particularly grubbing and moulding, might be implicated; or perhaps the agent may be a soil-inhabiting insect. Hecke (9), who developed a similar argument, has suggested, following de Bary (6), that perhaps some insect in its emergence may bring the fungus to the surface; and this surmise is not so far-fetched as it might seem. It is well known that the larvæ of certain flies commonly feed in blighted tubers, and the emergence of some of the perfect insects (*Sciara* sp.) from blighted tubers in a dish in the laboratory prompted us to try if they carried conidia, which they were found to do, sometimes abundantly, in four cases out of five (fig. 3). An infection experiment unfortunately failed, due, it is believed, to faulty technique.

Infection of Tubers of the New Crop in the Absence of Foliage Infection.

An experiment conducted in the open in 1925 to endeavour to secure aerial infection with blight from the planting of diseased tubers yielded a negative result, so far as the stalks and foliage were concerned, but revealed the fact that the new tubers could be invaded, although it was quite certain that no

disease whatever existed on the foliage. The blighted tubers had been planted in unmanured soil, which had not previously been used for potatoes, in a box with a partially open bottom, as described in the first experiment. As no blight appeared, although the plants were well grown, they were turned out on May 25th, when a single new tuber (1 out of 79) showed typical blight, which was confirmed by microscopical examination. The disease had involved all the basal end of the tuber, nearest the rhizome, in a quite symmetrical way, just as in the case of black leg or pink rot, and the conclusion was come to that the disease had entered through the stolon, which was decayed—to such an extent, unfortunately, that blight could not be recovered from it. As the tuber was detached, the mode of infection could not be exactly determined; but the fungus was still found to be viable, and capable of producing conidia, in some of the old tubers. Presumably, it could have grown out from this source through the stolon.

Brooks (2) has recorded the presence of infected tubers borne on non-infected stolons contemporaneously with the first appearance of foliage infection, and Löhnis (13) has reported several similar cases before blight appeared on the foliage, or in its entire absence. Some observations of Horne (10) and of Schander (24) are very similar; but it is obviously difficult to be sure, in the case of large areas, that no blight whatever was present on the leaves at any time during the season. In very hot regions, as one of us has observed in Central Canada, the blight sometimes appears on the foliage in early summer, and then, with the advent of very hot weather, disappears completely for the remainder of the season. MacAlpine (14) has also reported that, in dry hot years in Australia, the tubers may become diseased without any blight being visible on the foliage, and he assumes that the fungus grows direct from the "set" into the new tubers by way of the stolons. No proof of this, however, is available, apart from that now recorded.

Discussion.

Salmon and Ware (23) have conveniently summarised the work of the four investigators, who had, previously to themselves, recorded a connection between the first outbreak of blight and the emergence of shoots diseased *ab initio*, and we need not, therefore, repeat it. The work of the many investigators who failed to find such a connection is, however, not so well known, and it will be necessary for the purpose of this discussion to refer to it briefly.

De Bary (5), himself the originator of the diseased sprout theory, which was based originally on laboratory experiments, failed for fifteen years to confirm it, and during this period abandoned it more or less, for we find him considering the possibility of the direct migration of mycelium into the leaves at one time, and, later on, the question of heteroecism. It was not until 1876 that he (6) announced the confirmation of his original theory by finding diseased shoots in the open. Melhus (15, 16) has given a list of twelve investigators who failed to duplicate this experiment, and no useful purpose would be served by going over this ground, though some of the work is referred to in other connections, but the more recent negative results of Brooks (2) and Löhnis (13) may be added.

Melhus (15, 16) worked out for the first time the conditions favouring the emergence of diseased shoots, and showed that by raising the temperature to 23°–27° C. their growth was materially promoted. This we have been able to confirm by direct experiment, and the large disproportion of positive results in favour of the indoor trials in our own work, as well as in that of Melhus,

is also confirmatory. We look upon the experiments of Pethybridge (21, 22), and of Salmon and Ware (23), as pointing in the same direction also; for their outdoor trials, always conducted on a larger scale than the indoor ones, and in one case repeated during three seasons (22), invariably failed, while the greenhouse experiments succeeded. It is obvious that the temperature in a greenhouse would have been higher than that outside.[1] From the contradictory results secured in both cases, it would be at least as permissible to conclude that foliage infection out-of-doors arose in some other way than by the emergence of diseased shoots. At all events, in the light of experience extending over many years, and in view of the ease with which diseased shoots can be made to emerge under special conditions, we do not think much importance can be attached to indoor trials.

The situation, therefore, is that four investigators only in the last sixty-five years have been able to connect the first appearance of blight *in the open* with the emergence of diseased shoots, in spite of widespread search. The question next arises, if the majority of primary outbreaks in nature—events which may be assumed to be comparatively common, occurring as they do year after year, and probably in many fields throughout the country, largely in spite of the diversities of climate, season, and soil—can be traced to this source. The evidence in favour of this is certainly not strong; and the case for the alternatives must now be examined.

Jensen (11), Hecke (9), Clinton (4), and Brooks (2) have shown, with varying degrees of probability, that the first outbreak of blight on the leaves is not always associated with the emergence of shoots which are infected *ab initio*; and from the experiments of Hecke and Brooks it appears likely to us that the infection arose from conidia produced in the soil, although Brooks considered the intervention of some form of resting body to be probable. Jensen, although he found some shoots to emerge which were diseased from the beginning, also knew that few succeeded in doing so, and was inclined to emphasise the importance of secondary infections produced by conidia in the soil on the underground portions of shoots which had already come through the ground.

The production of conidia in the soil has been demonstrated by many workers (9, 3, 12, 16) since the time of de Bary (6), who considered that their formation was not very frequent, and that little importance should be attached to them, a view which was shared by Melhus (16). The work now reported on by the present authors, in confirming the occurrence of the fungus in the soil in the neighbourhood of diseased tubers and sprouts, emphasises how common this event is, and how long the fungus retains its vitality there, and demonstrates the fact that the new tubers may be infected direct from this source or from the parent plant. It is also shown that the fungus can spread independently in the soil under conditions approximating to those of nature, but only to a limited extent (about ¾ inch), under the conditions of these experiments, and for a limited period of time, so that it would probably be unable of itself to reach the surface of the ground from tubers planted at the usual depth. It is suggested that tillage operations, or the movements of soil insects, may transport the fungus to a position from which the foliage may be attacked, but no direct proof of this is available.

Where the blighted tubers are at or near the surface, the fungus could penetrate the upper layers of soil, and provide an abundant and comparatively

[1] Pethybridge (21), who conducted two experiments, one in a warm and one in a cool house, obtained a positive result only from the latter; but the number of plants (3) in the warm house was very small, and the maximum temperature reached (85° F.) was appreciably above the optimum for *P. infestans*.

lasting source of infection for stalks and leaves. This would be particularly likely to happen where decayed tubers are thrown to the ground around pits and other storage places, and remain uncovered, or only slightly covered, by the soil. The earliest outbreak of blight for the year 1920 was observed at Athenry, in Ireland, in the first week in June, on a plant coming up on the site of an old potato pit; a plant growing from a heap of discarded diseased tubers showed a very early primary outbreak of blight at Glasnevin some years later; and at least five similar cases have been recorded elsewhere.

To sum up the arguments in favour of this view—the infectivity to sprouts and tubers of the material produced around blighted tubers in the soil has been proved, and there is strong presumptive evidence that it may lead to blight on the aerial parts of the plant. This material occurs more commonly than do blighted shoots which succeed in reaching the surface, particularly under conditions approximating to those normally occurring out-of-doors in the earlier portion of the season. The fungus, as it occurs in the soil, retains its pathogenicity sufficiently long to account for its survival during the long interval which frequently elapses between planting time and the first appearance of blight—an interval which has never been satisfactorily explained, and which is particularly difficult to account for, if one relies solely on the emergence of diseased shoots as a source of infection. The arguments against this theory are the fact that there is no unequivocal proof of the connection between this material and foliage infection, and the difficulty which still exists of accounting for its conveyance from deeply-planted tubers to the surface of the ground.

If it could be proved that *P. infestans* persisted independently in the soil in the open, another alternative source of blight infection would be available (7, 8). No evidence is, however, forthcoming from our own experiments that the fungus can survive indefinitely in a vegetative condition, or can hibernate, in this way.

We have shown elsewhere (20) that two large lots of fertile clay soil from a blighted field of potatoes, which were proved to contain *P. infestans* in the previous autumn, were entirely non-pathogenic to potatoes when tested four times in the following spring. The soil had been kept out-of-doors, and evidently the fungus had died out during the winter. The same had been the case in earlier laboratory trials in 1920–21 (17), and in field trials (19), in which the longest proved periods of survival in the soil were 44 days and about 46 days, respectively. It is further shown in the present paper that, even when the mycelium again finds its way into the soil in spring (as, for instance, from blighted tubers), its development, both in time and space, is limited; and that, unless the tuber is very near the surface, another agent is probably necessary to bring the fungus into a position where it can produce aerial infection.

The only conclusion possible from our experiments in this connection is that the blight fungus exists independently in the soil of our potato fields (particularly the surface layers, but rapidly decreasing in amount below) in autumn, after blight has been severe on the foliage, but that it dies out after some weeks. Again, in spring, a second infection of the soil occurs in the immediate vicinity of blighted tubers, but this also is temporary, and even more limited in extent than before. These results were secured on fertile clay soil, but it appears probable from Miss de Bruyn's (8) later work that the behaviour of the fungus in soil very rich in humus (such as peat) might be different; and, perhaps still more probable, that hibernation may take place in manure. The same difficulty again is met with there, as to how the infection reaches the surface of the ground.

It is shown by one of us (18) elsewhere that *P. infestans* has not entirely lost the power of producing its sexual organs on the potato plant in nature.

These bodies, which occur on the surface of the tubers, and have been found in the soil surrounding a blighted tuber under conditions approximating to those of nature, consist, as a rule, of oogonia without antheridia, in a small proportion of which apparently perfect parthenogenetic oospores mature. The antheridia were also found to occur, but only very rarely, in cultures on portions of tubers *in vitro*, resulting in perfect sexual spores. While the spores are apparently not common, and their power of perpetuating the fungus has not yet been proved, they constitute another potential source of infection, the importance of which it must remain for future work to determine.

SUMMARY.

Two further cases are recorded in which diseased shoots produced by blighted tubers planted in the open reached the surface of the ground, and led to aerial infection. Similar successful results were secured in the laboratory when the experiments were carried out at 25° C.

In laboratory experiments at lower temperatures, the fungus was directly observed in many cases to penetrate the sprouts, which, however, did not reach the surface; and to spread into the immediately surrounding soil, where its pathogenicity was retained for a limited period—in one case extending to 57 days, at least. This is believed to represent what usually happens in the field, but the problem of how this material reaches the surface from deeply-planted tubers has not been solved.

The emergence from blighted tubers of flies of the sp. *Sciara* carrying conidia of *P. infestans* is demonstrated under laboratory conditions.

The infection of a tuber of the new crop produced by a plant from a blighted tuber, in the entire absence of foliage infection, is demonstrated.

The relative importance of the following factors in causing primary foliage infection is discussed: (1) the emergence of diseased shoots; (2) the production of infecting material in the soil in the neighbourhood of blighted tubers; (3) the possible hibernation of the fungus in the soil in the open; and (4) resting-spores, which are shown elsewhere to occur sometimes on blighted tubers under natural conditions.

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DESCRIPTION OF PLATE XIX.

Fig. 1.—Growth of *Phytophthora infestans* into sprout (S) and soil (M) from blighted whole tuber planted in soil. Note scanty root development.

Fig. 2.—Normal root development from healthy whole tuber under same conditions, to compare with fig. 1.

Fig. 3.—Conidium of *P. infestans* among bristles on leg of fly of sp. *Sciara* which emerged from blighted tubers in laboratory.

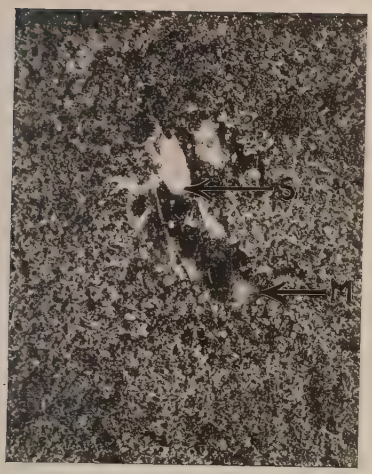

FIG. 1.

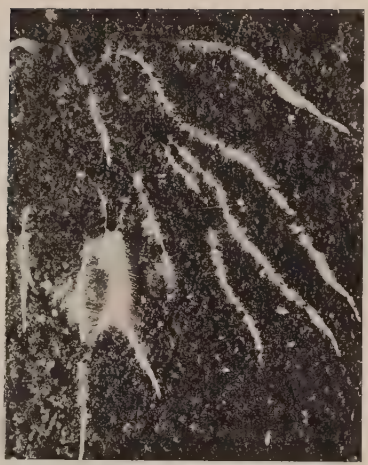

FIG. 2.

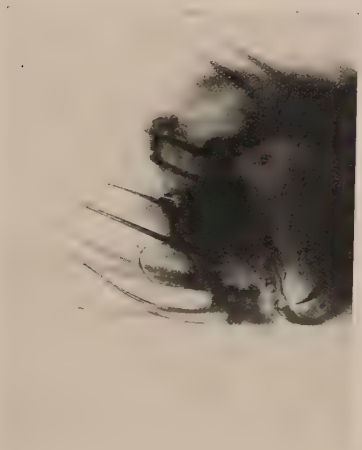

FIG. 3.

No. 36.

ON THE INDEX FOSSIL OF THE *CLEISTOPORA* ZONE.

By LOUIS B. SMYTH, M.A., Sc.D.,
Lecturer in Palaeontology, University of Dublin.

(Plates XX–XXII.)

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When collecting at Hook Head, Co. Wexford, recently, I obtained a considerable number of specimens of the coral, usually known as *Cleistopora* aff. *geometrica*, used by Vaughan as the index of his "K" zone. In comparing the new material with specimens from the S.W. Province, it was found that the species needed more accurate description. It turns out to be, not a *Cleistopora*, but a *Vaughania*, and is here given the name *V. vetus*.

CLEISTOPORA GEOMETRICA Ed. and H.

In the year 1851 Milne-Edwards and Haime (1) described and figured a coral from the Lower Devonian of Viré et Loué, Sarthe, France, under the name, *Michelinia geometrica*. Their description is as follows:—

"Corallum flat, free or encrusting; calices of the same specimen equal, and in the form of regular hexagons, of very slight depth; the uppermost tabula, which occupies the bottom of them, almost horizontal, and strongly granular on the surface. It appears to have 28 vertical septal striae. The diameter of the calices in different specimens varies from 5 to 7 mm., or is even a little greater."

Nicholson examined specimens from the Devonian of Viré, and published in 1888 (2) an account, accompanied by drawings of horizontal and vertical sections. According to this author, the base of the corallite is filled with trabeculate tissue, the upper surface of which gives the granular floor of Edwards and Haime,[1] and "'mural pores' are only represented by vermicular and tortuous tubes." In his figures of sections, no tubes are seen passing through the walls. Even if the tubes are tortuous, one would expect to see some sections of them on the way across.

The "K" Zonal Index.

The fossil chosen by Vaughan as index of his "K" zone was called by him *Cleistopora* aff. *geometrica* (Ed. and H.), (3, pp. 189 and 268). He describes it in 1903 (4) as agreeing in all the main characters with Edwards and Haime's definition and figures. He adds: "the calices average 4 to 5 mm. in diagonal, and are usually almost perfect hexagons; the walls are thick and granular, but, when well preserved, show a thin central ridge along their middle line;

[1] It is hard to see how the tissue figured by Nicholson would produce the appearance of isolated circular granules shown in Edwards and Haime's figure.

there are four or five grooves running across each wall in all those specimens which have been worn down by weathering (these grooves represent tubular channels through the walls); the floor is almost horizontal and granular. (The granulation of walls and floor indicates clearly the trabecular structure of the vesicular tissue, the possession of which characterizes the genus *Cleistopora*.)''

It may be mentioned here that, though the walls and floor *are* granular, sections reveal the complete absence of vesicular tissue and trabeculate structure from this organism (*vid. infra*, p. 426), and, therefore, it cannot be placed in the genus *Cleistopora*.

As it is identical with the supposed types of *Astreopora antiqua* M'Coy, we shall now consider that species.

ASTREOPORA ANTIQUA M'Coy.

M'Coy, in 1844 (5), described and figured a fossil from Hook Head, Co. Wexford (6), under the above name. His description is as follows:—'' Discoid, convex; surface with large circular cells, in quincunx, about one-third their diameter apart; sides of the cells radiatingly striated; intervening flat spaces, with minute, irregular, curving ridges.'' ''Diameter of masses about one inch, diameter of cells one line.'' He notes its resemblance to *Hydnopora cyclostoma* Phil.

There are in the Griffith Collection in the National Museum of Ireland three specimens from Hook Head, which purport to be the types. They are much weathered and poor (Pl. XXI, figs. 4, 5, 6). M'Coy's figures, however, are not representations of any of these three, though they *may* have been based upon them. The description does not agree with the specimens, and, indeed, not too well with the figures. The description says: ''Cells . . . about one-third their diameter apart.'' In one figure this proportion is at least one-half; in the other, one-quarter. The diameter of the ''cells'' is said to be ''one line,'' i.e., 2·1 mm. In the specimens, the internal diameter is 2·5 to 3 mm. (3·5 to 4·5 measured from centre of wall). The '' cells '' are all '' circular '' in description and figures. A few of the weathered calices might be so described, but the most casual glance shows that most of them are angular. '' Sides of the cells radiatingly striated'' is a feature not a trace of which can be seen in these specimens, though such a description might, perhaps with some propriety, be applied to unweathered material from Hook Head (see Pl. XXI, fig. 2b).

The circularity of the calices and their striation are characters of *Microcyathus* (*Hydnopora*) *cyclostoma* (Phil.), which also occurs at Hook Head, though not recorded by M'Coy, unless it be the species under consideration.

In view of the above facts, M'Coy's species must, unfortunately, be rejected.[1] In memory of the name thus laid to rest, I propose to call the species ''*vetus*.'' As it is evidently very closely related to *Vaughania cleistoporoides* Garwood, I include it in the same genus.

VAUGHANIA VETUS sp. nov.

Cleistopora geometrica A. Vaughan, Proc. Bristol Nat. Soc., 1903, N.S., **X**, Pt. II, p. 99, pl. I, fig. 3.

Cleistopora aff. *geometrica* A. Vaughan, Quart. Journ. Geol. Soc., 1905, **LXI**, p. 268.

? *Astreopora antiqua* F. M'Coy, Synop. Char. Carb. Fos., Ireland, 1844, p. 191, pl. 26, fig. 9.

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[1] The generic name *Astreopora* Blainville, 1830, would not be applicable on any interpretation of M'Coy's species (see H. M. Bernard, Brit. Mus. Cat. Madrep. Corals, vol. ii, 1896, p. 81).

Holotype.—The specimen figured by Vaughan, 1903 (*loc. supra cit.*), from the K_2 sub-zone of the Avon Section, Bristol, in the Stoddart Collection, University Museum, Bristol.

The following description is based on material from the Bristol district in the British Museum, in the Bristol University Collection, and in the collection of Dr. Stanley Smith, supplemented by my own collection from Hook Head. Reference numbers, preceded by the letter R, given in brackets, throughout the description, are those of British Museum specimens, illustrating the various points.

The corallum may be regarded as consisting of a basal plate, upon which stands a network of walls, surrounding calices. It tends to be circular in outline.

Attachment.—It is generally attached to some foreign object. In most cases observed, this has been a Brachiopod valve, at least six genera having been noted, but other supporting bodies also occur. A specimen from Hook Head encrusts a conical body, perhaps an Orthoceras fragment, and has developed most irregularly. The encrusting colony often extends far beyond its support (R 22507, and Pl. XX, figs. 5 and 6). In many cases this is quite a small body—a colony of 24 mm. diameter, for example, having as support a triangular fragment of shell of 3 mm. side. Sometimes no trace of a supporting object, or its impression, is visible on the fully exposed under-side.

Under Surface.—Where not in contact with a support, the under surface is seen to bear sharp, irregular, radial ridges (R 22507), which vary much in strength in different specimens. Pl. XX, fig. 6, shows a coarse-ribbed one from Hook Head; fig. 5, a fine-ribbed form from Bristol. Equally fine-ribbed Hook specimens occur.

Besides these sharp radial ridges, the under side has irregular, concentric, rounded ridges, like the epitheca of many Rugose corals (R 22507, Pl. XX, fig. 5).

Corallites.—The largest specimen I have seen has 37 corallites (Pl. XXI, fig. 12), and it is not complete; but more than 20 is the exception. The corallites vary in diameter from 3 mm. (R 2699, and Pl. XXI, fig. 10) to 7 mm. (Pl. XXI, fig. 9). Commonly, those in one corallum are much alike in size, but there are exceptions. Their shape varies from circular (Pl. XXI, fig. 1, central corallite) to sharply polygonal. Those nearest the centre commonly have their inner (proximal) margins curved, while the outer (distal) margins are angular (R 22504, and Pl. XX, fig. 2); those farther out are generally angular on all sides. They are extremely shallow, with flat, or concave, floors.

The floor and lower slopes of the wall are often seen to be covered with minute, close-set, rounded granules (R 16472, and Pl. XXI, fig. 2a), but in many specimens they appear perfectly smooth, probably owing to the state of preservation. The granulation seems to be an original structure, and not due to silicification. In many cases there is a more irregular granulation, obviously due to beekite.

Walls.—The walls are highest in the central part of the corallum, 2 mm. being the greatest height observed. Approaching the margin of the corallum they begin to decrease in height, until they reach base level at the periphery. They are also thickest—up to 4 mm. across the top—in the central region (R 22504, Pl. XX, fig. 1; Pl. XX, fig. 2; Pl. XXII, figs. 3b, 3c), where they have a flat, or slightly concave, upper surface, and become thinner outwards, until the top is a sharp ridge.

The flat, or concave, top of the wall is ornamented with a pattern of raised granules, often running together into short, wavy ridges, which tend to be at right angles to the sharp margins of the calices (R 22504, and Pl. XX, figs. 1, 2). These ridges produce a beaded appearance on the calyx margins. They may be continued some way down the lateral slopes of the walls.

The lateral slopes of the walls vary from vertical, or even overhanging, to a gentle incline. Overhanging walls (R 22504), if present, are usually on the side of the corallite which is nearest to the centre of the corallum.

The broad wall-top frequent in the centre of the corallum is generally associated with overhanging walls in the contiguous corallites; hence, in weathered specimens, where the wall-top is destroyed, the walls appear of normal thickness (compare figs. 1 and 2 with 3 and 4, Pl. XX).

Canal System.—On the inner surface of the wall, near the floor, is a circular canal running round the corallite (Pl. XX, figs. 3, 4). This may be an open groove (R 124, and Pl. XXI, fig. 2b), or a tunnel in the substance of the wall. Often it is a groove for part of the circuit, and sinks beneath the surface for the rest of the way (Pl. XXII, fig. 1n). Where it lies below the surface it communicates with the cavity of the corallite by an irregularly spaced row of circular to elongate pores (Pl. XXI, fig. 1). In some cases the ring appears to be incomplete. Marginal corallites have the ring canals on the inner side only.

The ring canals of adjacent corallites are connected by generally straight connecting canals, rather irregularly spaced (R 16472, and Pl. XXII, fig. 2).

Above the level of the ring canals there are direct canals, usually straight, running through the walls from one corallite to the next (R 22503, and Pl. XXII, fig. 1p).

These are often exposed as grooves owing to the destruction of the top of the wall by weathering (Pl. XX, fig. 3), but similar grooves may sometimes be seen crossing the top of the wall in apparently perfect specimens (R 22504, Pl. XX, fig. 1).

Marginal Corallites.—The marginal corallites have a wall, which is highest on the inner side, gets gradually lower at the sides, and is absent from the outer side. This outer side is a lobe of the basal plate, or continuation of the floor of the corallite, with an arcuate, serrate edge. It is marked on its upper surface by radial grooves, separating simple, or forked, ridges, each of which ends in one of the serrations (Pl. XXI, fig. 1). These floor ridges correspond to the ridges on the under surface, already described, which, therefore, also end in the serrations.

Microscopic Structure.—The substance of the corallum is compact and fibrous, the fibres being at right angles to the surfaces. The Bristol district specimens examined are considerably mineralised, but the fibrous structure is clearly visible in the less altered parts (R 16472, Pl. XXI, fig. 3). The Hook material is strongly silicified, but even here the fibrous structure can be often made out. In none of the specimens from either locality could the arrangement of fibres in relation to the canals be satisfactorily determined.

G. J. Hinde states (7) that he examined examples of *Astreopora antiqua* "from the same locality as the type," "which correspond with M'Coy's figures." He says, "the walls apparently consist of compact tissue, in which there are some irregular tubes—as if due to some boring organism."

Development.—The youngest corallum I have seen (Pl. XXII, fig. 3a) is a rather weathered specimen with two corallites, "parasitic" upon an older colony. The common wall sends out two cusps on each side, which partially embrace the calices. Beside the common wall in each calyx is a small embank-

ment of tissue, between which and the wall is the first arc of the ring groove. The outer borders of the corallites have been destroyed by weathering.

The next two corallites to appear (3, 4, Pl. XXII, fig. 3d) form a cross with the first two (Pl. XXI, fig. 8). Young ones then develop in the angles between the first four (5–8, Pl. XXII, fig. 3d; Pl. XXI, fig. 7, right). There seems to be a tendency to this symmetrical development (Pl. XX, fig. 2), but irregularities supervene sooner or later. In Pl. XXII, fig. 3b, for instance, 5 and 6 have appeared before 7 and 8; and in 3c, although 7 has not yet developed, 6 is quite large, and another one (9) is just forming between 6 and 2.

Thus the walls gradually creep out, following the extension of the basal plate; at intervals their free ends fork, and a new corallite appears between the branches.

In the early stages (Pl. XXII, fig. 3) the appearance of the wall system in plan quite suggests a mountain mass dissected by cirques, the varying thickness of the flat-topped, central walls corresponding to the varying amount of original mountain surface left by recession of the cirques, and the thin, sharp-edged, outer walls to the arêtes.

As the walls are built up, direct connecting canals (radial canals) are left piercing them. A ridge of tissue appears on the calyx floor parallel to the wall, and, along with the wall, forms a ring groove. With further growth this may be partially, or entirely, roofed over, becoming a ring tunnel, but pores are left, connecting it with the calyx. The first-formed, lowest radial canals connect the ring canal of one corallite with that of the next. As the walls get higher other radial canals are developed at a higher level, each first appearing as a groove on the top of the wall, and the wall tissue subsequently closing over it. The grooves on the wall top, which are destined to become radial canals, are not the grooves separating the granules and ridges already mentioned. The latter are considerably finer, and more irregular, and are purely a surface feature.

WHY WAS *VAUGHANIA VETUS* CONFUSED WITH *CLEISTOPORA*?

The Bristol specimens are often mineralised in irregular patches, so that in section there are interlocking, map-like areas with three different appearances—opaque, milk-white; transparent, colourless; and translucent, greyish—their distribution being quite unrelated to structure. Add to this the pattern produced by the plane of section cutting the irregular canal system, and the fact that parts of the canals are occupied by matrix, whilst other parts are infilled with crystalline deposit, and it will be understood that a quite "trabeculate" appearance can be produced.

And there is no doubt of the considerable resemblance to Edwards and Haime's figure, referred to above, p. 423.

But I have never seen in *V. vetus* any appearance of *the base of the corallite* being filled with trabeculate tissue, as described and figured by Nicholson in *Cleistopora* (see above, p. 423).

COMPARISON WITH *VAUGHANIA CLEISTOPOROIDES* GARWOOD (8).

It will be noticed that Prof. Garwood states that his species "closely resembles the form from the Avon section, referred by Dr. Vaughan to *Cleistopora* (*Michelinia*) *geometrica* Ed. and H." He does not criticize Vaughan's identification, but goes on to point out that his species differs from that genus, *as described and figured by Nicholson,* in the following points:—

1. The absence of the trabecular structure which characterizes that genus.

2. The presence of compact fibrous coenenchyma, forming the whole of the corallum.
3. The presence of a definite system of ring-canals and branches.
4. The presence of a well-developed basal epitheca, which is unattached.

Now, these four points (with the qualification that the corallum may be attached *or* unattached) all apply to Vaughan's species, and, therefore, separate it from the true *Cleistopora geometrica* Ed. and H., and assimilate it to *Vaughania*.

In order to complete the comparison with the latter genus, I may mention one or two details not referred to in Prof. Garwood's description.

The corallites of *Vaughania cleistoporoides* are sometimes rounded, and the walls bounding them may have flat, or slightly concave, tops, bearing the same type of ornament described above for *V. vetus*. This ornament is very subdued in the specimens I have seen. It is present in the specimen figured, Pl. 48, fig. 7 (8), though it does not show up in the photograph. The slight shadow, due to one side of the concavity on top of the wall, can just be made out in places on the left of the figure, and the beaded calyx-rims on the extreme right.

Radial ridges ending in teeth occur on the peripheral parts of the floors of marginal corallites. This feature again is very rounded and subdued.

There can be no doubt that *V. vetus* and *V. cleistoporoides* should be included in the same genus.

Differences.—The Bristol and Hook species has radial ridges on the under surface; its circular canals are to a great extent unroofed; it is *usually* attached; its corallites vary much in size (from 7 to 3 mm.).

Vaughania cleistoporoides shows no radial ridges on the under surface; its circular canals are never open grooves, but only closed tunnels; it is apparently free; its corallites are very constant in size, at about 4 mm. (I am indebted to Prof. Garwood for confirmation of these points.)

Comparison with *MICROCYATHUS CYCLOSTOMA* (Phil.).

This agrees with our species in having a surface between the margins of adjoining corallites, with somewhat similar ornament; but differs in that this surface extends between all the corallites, and not merely the earliest formed. It also differs in the circularity of its corallites, their greater depth, and the marked striation of their inner slopes.

The floor of a corallite consists of a plate of compact tissue, fibrous at right angles to its surface, just as in *V. vetus*. This layer is continued up the sides, to form the wall of the calyx, but is here pierced by pores. Separating the wall layer of one corallite from that of the next, and also surrounding the corallite on the free side, is "trabeculate tissue," consisting of fibrous material, just like the wall, enclosing spaces continuous with the pores, and forming a complicated system. But no trace of an arrangement resembling ring canals could be made out; neither are there any direct canals passing from one corallite to another.

Comparison with *MICHELINIA*.

The young corallum of *M. megastoma* has a considerable resemblance to *V. vetus*, but is distinguished by the absence of the flat wall-top, or distal surface, with its characteristic pattern; the wall, even in the earliest corallites, having a sharp top, which is the common margin of adjacent corallites. At an early stage, too, the corallites become deeper than any seen in *Vaughania vetus*, and exhibit several tiers of mural pores. Later, tabulae appear, structures

unrepresented in *Vaughania*. I have examined many specimens of young *Michelinia* spp. for ring canals. In most cases there was no trace of such a structure. But in a few cases the lowest tier of mural pores opened into a slight groove in the base of the wall. The specimens in which this was observed were highly beekitised, and the matrix had been artificially dissolved out. Moreover, the grooves only occur in some corallites of a colony, and none could be seen in otherwise similar colonies.

DISTRIBUTION.

Cleistopora aff. *geometrica*	... Bristol district (3)	K_1 K_2	
„ *geometrica*	... Westbury, Bristol (9)	K_2 Z_1	
„ aff. *geometrica*	... Mendip district (10)	K_2	
„ cf. *geometrica*	... Cardiff district (11)	K_1 K_2	
„ *geometrica*	... Pembroke and Tenby district (12)		γC_1
„ cf. *geometrica*	... „ „	K_2	
„ spp.	... „ „	K_1 K_2	
„ *geometrica*	... Dinant district (13)		
30–35 m. below *octoplicata* shales, correlated by Vaughan with			Z_1

At Hook Head, *Vaughania vetus* ranges throughout the sequence, which is apparently equivalent to Z–C_1. The fauna is not yet worked out, and it is uncertain whether any part of K is represented. But the top is certainly at least C_1, since *Cyathoclisia tabernaculum* Dingwall and *Caninia patula* (Mich.) occur some distance below it.

The relative horizons of the specimens figured are as follows:—

Loc. 13b close to top of series Cyathoclisia Loc. 17w Caninia patula	C_1
Loc. 30r „ 5a „ 4d „ 3h close to base of series	Z (?K)

It is hoped to publish details of the section at an early date.

ACKNOWLEDGMENTS.

My thanks are due to the Trustees of the British Museum, to the Acting Director of the National Museum of Ireland, and Mr. A. W. Stelfox, to Dr. W. D. Lang, and to Dr. Stanley Smith, for the loan of specimens; and to Prof. Garwood, for the gift of a specimen, and for much help in the study of *Vaughania cleistoporoides*.

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EXPLANATION OF PLATES.

Reference numbers preceded by the letters "T.C.D." are those of specimens in the Geological Museum of Trinity College, Dublin.

Plate XX.

Vaughania vetus sp. nov. × 2·5.

1. Railway cutting, Grovesend, Thornbury, Gloucester. (Brit. Mus., R 22504.)
2. Hook Head, loc. 17w. (T.C.D., F 131/1008.)
3. K_2 sub-zone, St. Monica's, Westbury-on-Trym, Bristol. (Dr. Stanley Smith's Collection.) Weathered specimen showing canals and pores.
4. Hook Head, loc. 17w. (T.C.D., F 132/1008.) Weathered specimen showing canals and pores.
5. Railway cutting, Grovesend, Thornbury, Gloucester. (Brit. Mus., R 22507.) Under surface.
6. Hook Head, loc. 17w. (T.C.D., F 133/1008.) Under surface.

Plate XXI.

Vaughania vetus sp. nov.

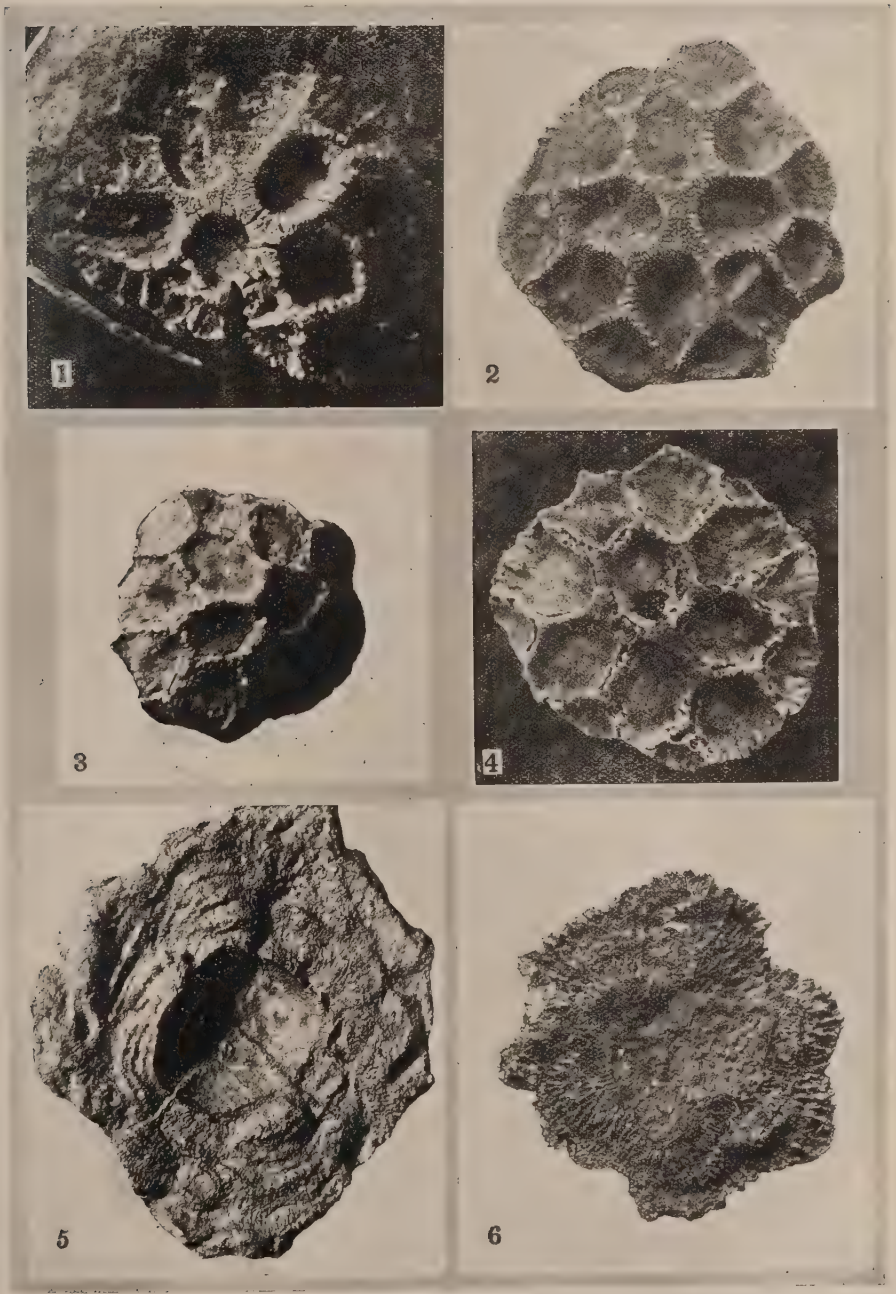
1. Hook Head, loc. 17w. (T.C.D., F 134/1008.) × 2·5.
   Shows a nearly circular corallite and narrow wall-tops. The more normal, wide wall-top is represented in the younger "parasitic" colony delimited by the white line. Note the pores with tumid lips leading to the ring canal in the circular, and some neighbouring, corallites.

2. Hook Head, loc. 17w. × 2·5.
   a. (T.C.D., F 141/1008.) Granular floor well displayed in marginal corallite with worm-tube.
   b. (T.C.D., F 142/1008.) Uncovered ring canals in an unweathered specimen.
3. K_2 sub-zone, Avon Section, Bristol (Brit. Mus., R 16472). × 10. Part of vertical section (b of Pl. XXII, fig. 1), showing fibrous structure. In the top left corner two white patches are parts of the supporting shell fragment. Above it is a section of a corallite wall with ring canal and pore, infilled with dark matrix, and mural pore passing half-way through wall, with clear crystalline filling.
4, 5, 6. Hook Head. (Griffith Coll., National Museum of Ireland, Dublin.) × 0·7. The supposed types of *Astreopora antiqua* M'Coy.
7. Hook Head, loc. 17w. (T.C.D., F 135/1008.) × 0·7. Two colonies; that on the right in the eight corallite stage.
8. Hook Head, loc. 5a. (T.C.D., F 136/1008.) × 0·7. Colony in the four corallite stage on a valve of *Leptaena*.
9. Hook Head, loc. 17w. (T.C.D., F 137/1008.) × 0·7. Large corallites.
10. Hook Head, loc. 17w. (T.C.D., F 139/1008.) × 0·7. Small corallites.
11. Hook Head, loc. 4m. (T.C.D., F 138/1008.) × 0·7. Small corallites. On a valve of *Spirifer*.
12. Hook Head, loc. 17w. (T.C.D., F 140/1008.) × 0·7. Large corallum.

PLATE XXII.

Vaughania vetus sp. nov.

1. K_2 sub-zone, Avon Section, Bristol. (Brit. Mus., R 16472.)
   Camera lucida drawings of serial sections made from solid surfaces by reflected light. × 2.
   a–n. Vertical sections about $\frac{1}{10}$ mm. apart. A piece of shell forming the substratum is indicated in the middle region; and sections of concentric ridges are seen where the corallum spreads beyond its support.
   p–s. Horizontal sections from above downwards. In q, a ring canal begins to show on the right. In r, the lower corallite is a marginal one, and the section cuts the grooved floor below and the partial ring canal above.
2. Hook Head. Camera lucida drawing of horizontal sections of canal system. × 2.
   a. Loc. 30r. (T.C.D., F 143/1008.) The black represents the canal system and other spaces in the section.
   b. Loc. 4d. (T.C.D., F 147/1008.) The black represents the coral substance. At the rather straight boundaries on right and left the section passes into the supporting *Leptaena* valve.
3. Hook Head. Camera lucida drawings of young specimens. × 1.
   a. Loc. 17w. (T.C.D., F 146/1008.) Colony of two corallites "parasitic" upon an older one.
   b. Loc. 17w. T.C.D., F 145/1008.)
   c. Loc. 13b. (T.C.D., F 144/1008.)
   d. Loc. 17w. (T.C.D., F 135/1008.) This is the colony on the right of fig. 7, Pl. XXI.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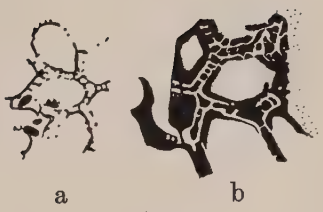

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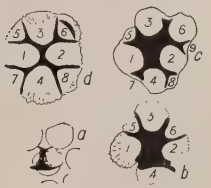

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No. 37.

THE SOLUBLE SILICATE CONTENT OF SOILS.

By W. R. G. ATKINS, Marine Biological Laboratory, Plymouth.

[Read April 26. Published separately May 9, 1927.]

In a previous paper (1924) the writer studied the electrical conductivity of extracts from soils of various types, and brought forward certain instances in which infertility was definitely connected with the absence of a sufficient supply of soluble salts, so that the 1 to 5 aqueous soil-extract approximated in conductivity to that of the water of an upland stream in a siliceous district. Mason and Wright (1925) have since shown that there is such a connection in the case of American cotton grown on various experimental plots in Nigeria, for the total number of open bolls per acre was in a large measure a function of the specific electrical conductivity of the 1 to 5 soil-extract. In the case of native cotton, on the other hand, the total number of open bolls was influenced not only by the salt content, but also by the water supply and by certain pests, for this cotton has a more protracted growing period. These soils with low conductivities are found in countries in which, at some time of the year, there is a heavy rainfall, though the conductivity method is of value, and has been largely used in detecting excess of salts also, such as found in rainless or irrigated regions.

In the more insoluble soils. however, silicates and alumino-silicates preponderate, often with considerable amounts of iron, as in laterite soils; the more soluble, on the other hand, are rich in carbonates or in organic substances. Clearly, then, a soil which deriving its bases from silicates shows only a small amount of soluble silica, and a small increment on standing, can only supply a crop with a meagre amount of the requisite bases. A determination of the silicate in solution may, therefore, afford a rapid method of detecting a soil of low fertility, just as the conductivity method may; but the silicate determination can be carried out with less costly apparatus, and with a consumption of but little more actual working time.

Furthermore, silicate is taken up by many plants in small amount, and its presence in solution in varying quantities from soil to soil may quite possibly in itself be a factor not altogether without significance. The writer was led to the study of the subject owing to the importance of the silicate content of natural waters for the growth of diatoms (1923, 1926).

The Estimation of Soluble Silicate in Soils.

Diénert and Wandenbulcke (1923, 1924) have provided an excellent colorimetric method for silicate. To 100 c.c. of the sample are added 2 c.c. of a 10 per cent. solution of ammonium molybdate,[1] followed by four drops of 50 per cent. (by volume) sulphuric acid. A yellow colour is produced, a maximum intensity being reached in about ten minutes, or rather longer with concentrated

[1] The molybdate should be kept in a resistance glass bottle, without a ground glass stopper, since the roughened portion is liable to give off silicate.

solutions. As a permanent standard, the originators recommend a solution of picric acid containing 36·9 mg. per litre. This, they state, corresponds to a silicate solution containing 50 mg. per litre of SiO_2. Thresh and Beale (1925) consider the method to be as reliable as the gravimetric, but prefer to take 40 mg. p.l. of picric acid, as equivalent to 50 mg. p.l. of SiO_2. The writer, however, made up his standard in 1923 according to Diénert and Wandenbulcke's proportions, and has, for the sake of uniformity, adhered to that in his analyses of natural waters and of soils. It is possible, however, to work with solutions far weaker than 50 mg. p.l. For natural waters, comparison is best made against standards equivalent to from 2–5 mg. for fresh water, and from 0·1–0·5 mg. p.l. for sea water. The limit, which can just be detected in a perfectly clear water, is 0·04 mg. p.l., using 100 c.c. in a Hehner tube and a 0·1 mg. p.l. standard. The tubes should have plane bases, both inside and out; one variety on the market has a curved surface inside, and is obviously not as accurate as the other. These are mounted on a Nesslerising stand, and viewed close together, so that the white opal plate is seen at the same time; this keeps the eye sensitive to the faintest yellow tint. Accuracy is increased by placing a white card, with lines ruled in ink, on the opal plate, so that half the field of each tube is covered by the card. The black lines apparently give the eye something on which to focus, otherwise it might focus somewhere inside the column of liquid. Both for work on phosphates, in which a blue tint is compared, and on silicates, the writer has found Hehner tubes preferable to either the Duboscq or Kober colorimeter for very faint tints, as a longer column is obtainable, also a direct comparison is made with a perfectly white plate. Extraneous light is cut out by a piece of cardboard held in front of the tubes. It has been shown (Wilson and Atkins, 1926) that phosphates only give the reaction in concentrated solutions. Owing to the minuteness of the amount of phosphate normally present in soils no error arises from this source, nor is any to be expected from arsenate.

Should the water or soil-extract have a slight tint, this can very often be matched against the standard picrate, calculated as silica, and a correction made. With deeper tints, recourse may be had to dilution—20, 10, or even 5 c.c. of soil-extract being made up to 100 c.c. with distilled water. The latter should be tested for silica, which it may contain if stored in a white glass vessel. Green glass Winchester bottles are usually quite satisfactory for storing distilled water for this analysis. With certain deeply-coloured extracts the method breaks down in its simple form, though it should be possible to precipitate the iron salts causing the colour, by adding a little ammonia, and afterwards to neutralise the filtered liquid and acidify it carefully with sulphuric acid. Too great acidity leads to a blue tint appearing in the yellow; the final acidity should be close to *pH*2. Alternatively, Wells' (1922) method for estimating silica in sea water may be used. An aluminium salt is added and then ammonia, to bring the solution to *pH*8. The silica is carried down with the alumina, which should be present in at least two molecular proportions for one of silica. The method avoids precipitation of calcium sulphate in the ordinary evaporation with acids; such precipitation gives trouble, as the resulting calcium fluoride is not all converted to sulphate, even when heated four times with sulphuric acid. Salts of aluminium give no colour with the silicate reagent.

The soils for examination were air-dried, and passed through a sieve of 100 meshes to the inch, and 10 g. of soil, correct to the first decimal place, was weighed out, and placed in a green glass bottle with waxed-cork stopper, together with 50 c.c. of conductivity water. The mixture was shaken at intervals for three hours. The turbid mixture was then cleared in an electric centrifuge, or allowed to stand till it settled.

The following examples suffice to illustrate the results obtained. All the samples had stood in the laboratory for several years in the air-dry condition. For comparison, the *pH* values are given, also those for the electrical conductivity at 0° C. It is possible that the duration of storage has some effect upon the solubility of the silicate, but the consideration of this has been left over:—

TABLE.

Silicate content of soils, reckoned as SiO_2, and phosphate content, reckoned as P_2O_5, both expressed as parts per million of air-dry soil; also electrical conductivity at 0° C. in reciprocal ohms $\times 10^6$ and *pH* value. Determinations made on 1 to 5 water extract; time of extraction, three hours.

Serial No.	Source of soil sample.	SiO_2	P_2O_5	$c \times 10^6$	*pH*
2	Disintegrating Archean rock, Worcestershire Beacon, Malvern ...	30	0·6	53	5·8
5	Douglas, Co. Cork, turnip field ...	34	1·6	110	6·0
6	„ „ second ditto, poor ...	18	3·3	93	5·8
118	Broadbalk field, Rothamsted, dunged ...	118	20	102	7·8
119	Ditto. unmanured	54	0·4	66	7·8
147	Soba, near Khartoum	124	0·8	High[1]	10·0

There is obviously nothing more than a general similarity between the variation in the soluble silicate and the conductivity. The great difference between the dunged and unmanured field is remarkable; it was suspected that the high phosphate content of the former might lead to an appreciable error in the silicate determination. Accordingly, a fresh solution of KH_2PO_4 was prepared, containing 0·1758 g. per litre, equivalent to 0·1000 g. p.l. P_2O_5. This gave a yellow tint, corresponding to only 0·3 mg. p.l. SiO_2 which appears to be genuinely due to the phosphate present, not to traces of silicate, since a blank with the distilled water and reagents gave 0·04 mg. p.l. SiO_2, or less, this being the limit that can be detected with certainty, though under good conditions a difference may perhaps be noted between 0·02 mg. p.l. and pure water. Atkins and Wilson (1926), when testing this point, gave 0·2 mg. p.l. SiO_2, instead of 0·3 mg., as above, but the new value appears to be the more accurate, as the lighting conditions were much better; the earlier determination, however, quite sufficed to rule out a source of error in water analyses for silicate, due to presence of phosphate. The determination was repeated, to allay the lurking suspicion that a phosphate solution must give a yellow colour with acidified ammonium molybdate. An old solution is very likely to do so, as it may contain appreciable amounts of silicate, derived from the glass; it is to be noted that a solution equivalent to 0·1000 g. p.l. P_2O_5 takes up less silicate than a more dilute one containing only 10 mg. p.l., due to the fact that the former is at *pH*5, the latter near *pH*6.

It was thought that the soils giving high *pH* values would probably give high results for soluble silicate also, but the few examples given lend no support to this view.

The writer's thanks are due to various friends, who kindly provided samples and information.

[1] Had 0·13 per cent. soluble salts.

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No. 38.

METHYLENE-BLUE (REDUCTASE TEST) IN MILK GRADING.

By M. GRIMES, M.Sc., Ph.D.; H. S. BOYD BARRETT, M.Sc.; and J. REILLY, M.A., Sc.D., University College, Cork.

[Read April 26. Published separately May 20, 1927.]

The quality of dairy products bears a direct relation to the quality of the milk supply from which they are manufactured. The grading of milk, as generally carried out, involves a fairly elaborate bacteriological and chemical examination, and the need for a simpler test has been generally felt. The ideal test would be one that could be carried out with the minimum of equipment, and in a short time, on a creamery platform. Barthel and Orla-Jensen have suggested the use of a dilute solution of methylene-blue, on account of the fact that most bacteria produce the enzyme "reductase," which decolourises methylene-blue. The greater the number of bacteria in the milk the larger the amount of reductase that will be present. Consequently, the shorter the time in which the methylene-blue is decolourised, working under standard conditions, the greater will be the bacterial count of the milk. All bacteria do not reduce methylene-blue at an equal rate, and, therefore, there is no direct relation between the bacterial count and the time in which the methylene-blue is reduced. If, however, the test is used for grading milk, the results generally will be approximately the same as those resulting from a bacteriological examination. This is especially the case when marked bacterial development has occurred in the milk. The test is now extensively used in many countries in the grading of milk, and is known as the "reductase" test.

In the test, a known amount of methylene-blue is added to a definite volume of milk. The standard mixture is prepared by adding to 40 c.c. of milk 1 c.c. of a dilute methylene-blue solution of known strength, obtained by diluting 1 c.c. of a stock methylene-blue solution to 10 c.c. (stock solution, containing 0·15 gram per cent. methylene--blue), or by using specially prepared tabloids, each tabloid giving 200 c.c. of standard solution. If a stock solution is diluted to 10 c.c. with water, and 1 c.c. of the dilute solution then added to 40 c.c. of milk, the final solution will contain 0·00015 gram of methylene-blue in 41 c.c. of solution, or one part methylene-blue to 273,300 parts of milk and solution of the dye.

The influences of impurities in the methylene-blue or variations in commercial grades of this product do not appear to have been fully investigated. It was considered desirable, before standardising such a test, to determine the composition of the various commercial brands of methylene-blue. From available information, it is not clear if it is necessary to use an absolutely pure methylene-blue in the test, and it is necessary before such a test could be strictly enforced that the influence of slight alteration in the test reagents should be known. In this connection, nine samples of methylene-blue obtained from different sources were examined as to their suitability for use in carrying out the "reductase" test. In the first instance, the samples were examined for actual methylene-blue content, percentage of moisture and inorganic residue, and the results are given in Table I.

TABLE I.

Brand.	Methylene-blue on the sample (without drying).	Moisture per cent.	Inorganic Residue (on ignition) per cent.
A	74·0	20·0	1·7
B	76·6	20·8	trace.
C	77·0	21·8	0·4
D	77·6	21·4	0·9
E	74·8	18·6	0·8
F	53·0	19·2	3·7
G	78·8	21·1	trace.
H	0·0	——	——
Tabloids	4·45	4·0	87·0[1]

One of the samples received (H) was found not to be methylene-blue, although labelled as such.

Samples A, B, C, D, E, and G are the hydrochloride of the dye base. The percentage of dye contained in these products (without drying) varied between 74 and 78·8 per cent. Sample F is a zinc chloride double salt with the hydrochloride of the dye base, and the dye content in this case was 53 per cent.

It will be seen from Table I that the percentages of moisture in the different samples approximate to 20 per cent. This figure is close to that required for five molecules of water of crystallisation. A dried sample of methylene-blue kept in a moist atmosphere for 12 hours again took up water to the extent of 21·8 per cent.

In most cases the percentage of ash is very low. Excluding the tabloids, the one brand which contained an appreciable percentage of ash was that which was low in methylene-blue content. The figure obtained (3·7 per cent.) does not indicate the total quantity of inorganic matter in this product, due to the inorganic constituents being partly volatilised during ignition. Traces of iron were found in practically every ash. Some tabloids of methylene-blue prepared by a Danish firm were also examined. The percentages of dye varied from 4·3 to 4·6 per cent. The moisture content was also low, the highest being 4·0 per cent.

The stability of dilute solutions of methylene-blue was next determined. When the percentage of methylene-blue in a given brand had been examined, a 0·15 per cent. solution of the pure dye was made up by weighing a suitable quantity (about 0·20 gram) of the commercial product making up to 100 c.c., or proportional amounts. If this solution is diluted 40 times with distilled water, the new solution will contain 0·15 gram of dye in 4,000 parts of solution. If 1 c.c. of this solution is now used with 10 c.c. milk, the final dilution is equal to 1 part of methylene-blue in 273,300 parts of the mixed solution of milk and dye. In order to test the stabilities of these solutions, the 0·15 per cent. solutions which had been made up for each methylene-blue product were kept in stoppered bottles, and tested from time to time. At the end of nine months no appreciable change of strength was found in any of the solutions. The solution obtained by diluting 1 c.c. of standard methylene-blue solution to 41 c.c. with distilled water is unstable. When tested the next day the dye content had appreciably lowered.

[1] This residue is mainly sodium chloride.

Experimental.

The dye was estimated by the method of Knecht and Hibbert, based upon the fact that methylene-blue is easily reduced by titanous chloride to a colourless leuco-compound. The reducing solution was stored in a large reservoir, and was kept continually in an atmosphere of hydrogen. A ferric iron solution of known strength was used for standardising the titanous chloride. From the iron equivalent of the titanous chloride the methylene-blue equivalent was calculated from the equation:

$$C_{16}H_{18}SN_3Cl + H_2 = C_{16}H_{20}SN_3Cl.$$
Methylene-blue.

It is seen that 112 gram iron are equivalent to 319·5 grams methylene-blue.

1 gram methylene-blue is dissolved in 250 c.c. water. 50 c.c. are measured into a conical flask and a small quantity of hydrochloric acid added. Carbon dioxide is passed into the flask, and the contents are heated over the Bunsen flame. Titanous chloride is then run until the blue colour just disappears.

The results obtained for the various brands by the above procedure were confirmed by estimating the dye with standard iodine and sodium thiosulphate. Methylene-blue forms a tetraiodo compound in the cold, which is insoluble in water, and is quantitatively decomposed by sodium thiosulphate. To 50 c.c. of methylene-blue solution excess of standard iodine is added. A brownish precipitate is formed, the blue colour of the solution being completely destroyed. Sodium thiosulphate is now run in until a very faint blue colour reappears. From the amount of thiosulphate added, the weight of iodine used to form the tetraiodo compound can be calculated. The methylene-blue is then estimated from the weight of the tetraiodo compound formed.

Comparison of the various samples of Methylene-blue used for the grading of Milk.

Since it was considered desirable to use the same strength of methylene-blue solution as is generally used in countries where this test has been adopted, two dilutions were made up from each sample of methylene-blue. One solution contained 0·20 per cent. of the commercial sample, equal to approximately 0·15 per cent. of methylene-blue (except in the case of the sample containing 53 per cent. of methylene-blue); which represents one part methylene-blue to 273,300 parts of milk and dye solution. The second solution contained 0·15 per cent. of the sample, equal to approximately 0·11 per cent. of methylene-blue (with the exception of the sample containing 53 per cent. methylene-blue). This would equal a dilution of one part methylene-blue to 372,700 parts of milk and dye solution. The average weight of the tabloids was 0·60 gram, and the average methylene-blue content, 4·45 per cent., equal to one part of methylene-blue to 307,100 parts of milk and solution of the dye. The stock solutions, which were in use for nine months, were found by chemical examination to be stable over this period. They were compared with a fresh solution, prepared by dissolving a tabloid in 200 c.c. of water.

The requisite number of dilute solutions were made from the stock solutions by dilution of one part of the stock solution to ten parts with water. In an experiment, to a sample of milk mixed and filled into sterile "reductase" tubes up to the 40 c.c. mark, was added 1 c.c. of the dilute methylene-blue solution. The contents of the tube were well mixed, and the tube (stoppered with sterile cotton wool) incubated at 37° C. The time was noted in which the blue colour

in the milk disappeared, or when (if the blue colour did not disappear evenly) the milk became white on mixing the contents of the tube.

Table II gives the results obtained in these experiments.

TABLE II.

Time in minutes during which a sample of milk became decolourised when standard Methylene-blue solution was added.

No. of Sample.	Tabloid Solution. mins.	All Solutions except Tabloid. mins.	Maximum Time (A) mins.	Minimum Time (B) mins.	A–B	p. c. Difference from mean time (nearest whole number).
1	20	20	21	18	3	15
2	65	66	71	65	6	9
3	78	80	83	70	13	18
4	95	95	98	95	3	3
5	140	147	155	140	15	10
6	277	278	293	268	25	9
7	327	346	390	320	70	20
8	355	360	375	335	40	11
9	380	375	420	350	70	19
10	385	387	395	380	15	4
11	395	441	465	395	70	16
12	407	409	443	365	78	19
13	445	452	460	445	15	3
14	538	550	585	530	55	10
15	552	530	555	485	70	13
16	750	765	803	740	63	8
17	827	852	892	802	90	11
18	870	852	890	803	87	10

It will be noted that the difference between the maximum and minimum times for decolourisation increases with increasing reductase time.

The test, however, is not sufficiently sensitive to distinguish between the two strengths of methylene-blue, or between the different brands of this dye. Even when tests were carried out in duplicate or triplicate, no definite correlation between times to decolourise was obtained. Experiments were next carried out to ascertain what variation in the strength of the methylene-blue solution was necessary to cause a variation in the time of reduction. The results are given in Table III.

TABLE III.

Variation in time of reduction of various strengths of Methylene-blue when added to milk.

	Time in minutes for samples of milk to decolourise.							
A	1	2	3	4	5	6	7	8
68,325 ...	225	213	208	435	408	343	440	493
136,650 ...	180	200	205	390	350	330	370	465
205,000 ...	160	195	200	340	320	320	350	440
273,300 ...	155	195	200	300	295	320	340	495
Tabloid Solution	150	183	192	—	275	307	340	432
341,625 ...	150	190	200	285	275	315	330	430
410,000 ...	145	185	195	275	270	300	330	420
478,325 ...	145	185	195	268	270	295	330	415
546,600 ...	145	180	190	255	270	285	330	405

The numbers in column A represent the ratio of one part of methylene-blue to parts of milk and solution of the dye.

A large variation in the ratio of methylene-blue to milk apparently does not alter the grade of the milk, particularly if the milk is reduced in less than two hours. This explains why the tabloid solution and the two solutions of methylene-blue did not differ markedly in the time taken for reduction when added to milk.

SUMMARY.

(*a*) Various samples of methylene-blue were examined, and found suitable for the reductase test.

(*b*) The stock solutions used in preparing the dilute solution required in carrying out the test were found to be stable over a period of nine months.

(*c*) The longer the reductase time the greater the variation between duplicate and triplicate tests.

(*d*) A large variation in the ratio of methylene-blue to milk apparently does not alter the grade of the milk, particularly if the milk is reduced in less than two hours. (See Table III.)

The authors desire to thank the Department of Lands and Agriculture for the various samples of methylene-blue used in the above tests.

No. 39.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1926.

[Read May 24. Published separately July 22, 1927.]

The quantity of emanation issued during the year was 13,480 mc., as compared with 14,401 mc. in 1925, and 12,885 mc. in 1924. No change has been introduced in the laboratory technique. The radium and the accessory apparatus have now been installed in their permanent positions in the new laboratory, the radium itself being stored in a fire-proof room. Detailed reports of the results of radium treatment sent in by two of the largest users of emanation are printed below.

REPORT FOR THE IRISH RADIUM COMMITTEE (ROYAL DUBLIN SOCIETY) OF RADIUM WORK CARRIED OUT IN THE YEAR 1926.

By Walter C. Stevenson, M.D., B.Ch., D.P.H.,
Surgeon, Dr. Steevens' Hospital, &c.

During the year 1926 radium treatment has been administered to 183 cases. Of these, 83 suffered from malignant disease and 100 from non-malignant conditions. The list of cases treated is shown on the next page.

The principal source of radiant energy for these patients was radium emanation, or radon, supplied in glass capillaries by the Irish Radium Committee of the Royal Dublin Society. This supply has been supplemented by 53·93 mg. of radium element sealed in 6 metal capillary cells.

Radon and radium capillaries were utilised with identical technique, and invariably in combination, when large doses were required in malignant disease. For protracted exposures in non-malignant cases two applicators, containing one milligramme of radium element each, have been found useful, and have often been combined with weak radon capillaries two or more weeks old. The great majority of the cases were treated as in-patients at Dr. Steevens' Hospital. Some of the malignant cases came from the country; others were kindly referred to me from other Dublin General Hospitals. These, when first seen by me, were inoperable, and mostly in advanced stages of the disease, with glandular involvement and other secondaries. In a number of cases the disease had recurred after operation. No malignant case has been refused treatment, and in all, the immediate effect of radium treatment is greatly appreciated by the patient on account of the rapid, if sometimes temporary, relief from pain and discomfort which radiation affords.

When the needs of all available malignant cases are satisfied—that is, they have received as much radiation as they can stand—the radiant energy is not wasted, but is used for non-malignant cases, either on ordinary surgical cases

TREATED WITH RADIUM, 1926.

183 CASES.

MALIGNANT, 83.

		Cases.
A. CANCER OF BREAST		5
B. CANCER OF UTERUS		9
Body	3	
Cervix	6	
C. CANCER OF MOUTH		23
Tongue	11	
Palate	6	
Tonsil	2	
Mucous Membrane (Cheek)	2	
Lower Alveolus	2	
CANCER OF PHARYNX		3
CANCER OF ŒSOPHAGUS		2
CANCER OF STOMACH		1
CANCER OF RECTUM		3
CANCER OF ORBIT		1
CANCER OF PENIS		1
CANCER OF LIP		4
CANCER OF SKIN		11
D. SARCOMA		12
Intracranial	3	
Maxillary Antrum	1	
Tongue	1	
Mucous Membrane of Lower Alveolus	1	
Pharynx	1	
Neck	2	
Uterus	1	
Vulva	1	
Humerus	1	
E. RODENT ULCER		8

NON-MALIGNANT, 100.

F. FIBROSIS UTERI		1
ABDOMINAL ADHESIONS (Painful)		3
Post-Dysenteric	1	
Post-Operative	2	
ACTINOMYCOSIS		1
ADDISON'S DISEASE		2
ADENITIS		2
CORNS		1
DEAFNESS		15
Chronic Hyperplastic Otitis Media	8	
Nerve Deafness	1	
Otosclerosis	5	
Senile	1	
DENTAL		1
ERYSIPELAS		1
GANGRENE		2
Inflammatory	1	
Senile	1	
INJURY		3
Chronic Pain	3	
KELOID		3
NAEVUS		3
PAPILLOMA		2
Tongue	1	
Palate	1	
PLEURISY		1
PROLAPSE RECTUM		1
PRURITUS		2
RHEUMATISM		3
SCARS		17
War Wounds	11	
Post-operative	3	
Tender	1	
Cut Nerves	2	
SCIATICA		2
SEPSIS		6
Acute	2	
Chronic	4	
SINUS		1
SPRING CATARRH		1
SYPHILIS		2
TONSILITIS		1
TUBERCULOSIS		15
Abscess	1	
Bladder	2	
Glands	1	
Lungs	2	
Lupus	5	
Joints	1	
Peritonitis	1	
Tubes and Ovaries	1	
Stomach	1	
ULCERS		6
WARTS		2

under my care, or at the request of my medical and surgical colleagues, for conditions which they appreciate have previously been benefited by this form of treatment. Most of these patients derive greater advantage by the use of small quantities of radio-active material applied for several days or weeks than by the massive doses necessary for malignant disease.

In this year's report the radiant energy used on the cases recorded is stated with the hope that it may serve a useful purpose. Such details are given as will enable some idea to be formed of the actual *dose*—in other words, the amount of radiant energy that acted in each case, and not merely the amount of energy that was dissipated during the treatment, the greater part of which is absorbed by filters, and in surface treatment wasted in the air.

The total energy available in each treatment has been recorded in *med.*, or millicuries disrupted during treatment. This is three-quarters per cent. per hour of the activity of the agent at the beginning of each hour. In the case of radium element this is for all practical purposes constant, and with the supply of 53·93 mgm. Ra. is taken as 0·4 med. per hour. The med. of radon, or its loss of activity per hour, is best ascertained from an emanation table, which gives the hourly activity of any quantity of radon for any period. The table I use constantly was compiled from Rutherford and Bragg's works, to save trouble in calculation. The advantage of the "med.," a term which is also employed by the French, is that there can be no ambiguity, and the uncertainty whether a writer means a radium salt or radium element or emanation in equilibrium with either does not arise.

As 3 med. is the equivalent of 400 mgh. or mch. (milligramme-hours or millicurie-hours), the method of recording the radiant energy used up can easily be converted. When the length of exposure is stated, the average activity of the agent employed can obviously be found by dividing the mgh. or mch. by the number of hours the agent was in use.

The principle of the technique I employ has been described in various papers, more particularly in the reports for 1924 and 1925. In the records below the method of treatment and the data which influenced the *dose* or the actual amount of radiant energy which acts upon each case is indicated by abbreviations, which I now explain :—

A. = Area.
A.A. = Area of Applicator in cms.
A.R. = Areas radiated.
R.A. = Radiating area.
B. = Boxes of Brass 3 mms. thick; radiating area 3×2 cm.
D. = Dose which acts on the patient, and varies in accordance with R.A., F., and d.
S.D. = Skin or Surface Dose.
B.D. = Buried Dose in tubes or needles inserted in malignant growth.
I.D. = Internal Dose in natural cavities of the body—as mouth, uterus, &c.
d. = Distance in mm. of radium from nearest body cell.
F. = Filter, thickness in mms. of brass, unless otherwise stated.
P. = Positions of needles or tubes when moved during B.D. or I.D.
R.C. = Radium Cells. Five of these each contain approximately 10 mgs. of radium element in platinum cells, 19 cms. long, 0·2 thickness of wall, 1·1 external diameter, the radium occupying about 15 mm. One cell of the same dimensions in steel containing 5 mgs. Ra.
R.N. = Radium Needles. 0·4 mm. steel; external diameter, 2·1 mm. Capable of containing 1.R.C., or Radon glass capillary.

S.N. = Serum Needle. Walls, 0·2 mm. steel. Capable of containing one radon capillary. External diameter, 1·1 mm.

T. = Tubes made of brass. 1.T.4, 2.T.15, 3.T.10, &c., indicate that the brass tubes have walls of 1, 2, or 3 mms. thickness; and that the external diameter is 4, 15, or 10 mms., and that the tubes are of such an internal length (2·2 cm.) as will contain varying numbers of either R.C. or radon capillaries, or both, at the same time.

S.T. = Surface Tubes. 1·75.T.5.

Each radon capillary, or radium cell, is a cylindrical source of radiation, with a diameter of 0·5 to 0·7 mms. and a length of 15 to 20 mms. The advantage of the capillary system is that it is as well adapted to surface radiation as for internal or buried treatment. The surface tubes in my work have practically replaced surface boxes, except in special circumstances, and when the capillaries have become too weak to be separated with advantage in surface tubes. For surface treatment, except when otherwise stated, surface tubes are placed in a row, parallel to one another, on a piece of felt 15 to 20 mms. thick, 5 cms. broad, and of the required length to suit the case. The distance in these cases is 15 to 25 mms. The dose per square millimetre is very easily ascertained. If, for instance, 16 surface tubes, containing, say, 180 millicuries, are placed in apposition with one another, each S.T. being 5 mms. in diameter, the radiating area will be 8 × 2 cm., and a dose of 1 med. per square cm., my usual S.D. in malignant disease, would be 16 med. This, on consulting the table, will require an exposure of 13 hours, as in that time 180 mcs. is reduced to 164 mcs. If the S.Ts. are spread out to 20 cm., the dose will be 40 med., which by the emanation table will require an exposure of 33 hours. For buried dose, radium needles are most frequently used. The needles, made of the required length by extension pieces, are inserted parallel to one another, and pulled out 2 or 3 cms. at the required intervals, till the whole area has been systematically radiated. In large growths several radon capillaries, sometimes with radium cells in the same tube, are placed in 1.T.4, 1.T.5, or other suitable tubes, and by means of wire extension pieces dealt with in the same manner. Usually only one tube in different positions is used for the internal dose—for instance, when treating the œsophagus, rectum, or uterus.

The following is an account, necessarily brief, of all the cases treated during the year, with the doses they received. Such particulars as involvement of glands are not particularly mentioned, but their absence has always been noted, except in the cases of skin cancer and rodent ulcer, which may usually be considered as operable. As operation would necessitate more loss of tissue and probably more disfigurement, radium treatment is the method of choice in dealing with these conditions, and would appear to be a more certain way of eradicating the disease.

Malignant Conditions. (83 Cases.)

A.—*Cancer of Breast.* (5 *Cases.*)

Case 1.—Right breast removed August, 1924. X-ray treatment immediately after operation, and three months later. First radium treatment in April, 1926. Surface application only. Considerable œdema of arm at the time. Surface radiation again, July 21st to 3rd August, continuously, as in some areas malignant nodules had not completely disappeared. Patient later got metastases, with kyphosis, at second lumbar vertebrae, which caused intense pain on the slightest movement. Treatment undertaken at the express wish of

the patient, as pain was intolerable. Relief of pain was immediate and marked, and the treatment greatly facilitated the nursing of the patient. Breast and lumbar spine again treated in December, 1926. Patient gradually lost ground, and died early in February, 1927. Her right arm was weak, but there was no sign of œdema. Beyond a small ulcer on the chest wall, there was no sign of disease in the breast area.

17*th to* 21*st April—S.D., Axilla, chest, R. neck, back and front shoulder,* 150·8 *mcd.*

21*st July to* 3*rd August—S.D., Axilla, back and front chest and shoulder,* 125·6 *mcd.* (53·93 *mg. Ra.,* 13 *days*).

5*th to* 7*th October—S.D., Lumbar spine,* 83·8 *mcd.*

15*th to* 17*th December—S.D., Lumbar spine, chest, and axilla,* 88·2 *mcd.*

Case 2.—Complete removal of breast by Halstead method in October, 1924. Treated for recurrence in October, 1925, in the scar, with glands in the axilla. The glands and nodules disappeared. She was treated again in May, 1926, for a further recurrence in a slightly different area. In each case the growth melted away, occasionally leaving an ulcerated area, which took some time to heal. The patient maintains her general health, and is able to work, and the recurrence seems to have been kept in check by treatment.

9*th to* 13*th October,* 1925—*S.D.,* 155·4 *mcd.*

10*th to* 13*th May—S.D.,* 153·0 *mcd.*

Case 3.—Aged 66. Had a tumour of the right breast near the nipple, and a weak heart. Needles were used in the growth, with the idea of giving an excessive dose, and subsequently removing the tumour under a local anæsthetic. The removal was refused by the patient. Five months later the tumour had completely disappeared, as had the glands in the axilla, which had also been radiated. Apparently cured (April, 1927).

20*th to* 25*th May—B.D.,* 21·1 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R.* 52¾ *hours. Area of growth* 7 × 8 *cm. S.D.,* 45·0 *mcd.*

Case 4.—Tubes and needles were inserted in this patient immediately after the operation, in which the surgeon removed the breast and glands, but not the pectoral muscles. There was a good deal of breaking down a couple of months after the radium treatment, and some septic infection. Six months later, there are no evident signs of recurrence, but there is great œdema of the arm and discharge from the axilla.

19*th to* 23*rd August—B.D.,* 89·6 *mcd.,* 9 *tubes* (6.*S.T.,* 2.*T.*8, 2.*T.*10), 33.*A.R. Three Ts. in Axilla, three Ts. in front, and three Ts. behind pectoral muscles.*

September and October—S.D., Chest, back, and front shoulder, 93·8 *mcd.*

Case 5.—The whole of this patient's right breast was extensively involved and irregular from subcutaneous nodules. Glands in axilla were present. On account of the extent of the involvement it would have been impossible to remove the breast without such sacrifice of skin as would render immediate covering by skin-flaps impossible. After treatment with buried tubes and radium needles, it was found impossible to remove the breast on account of the constitutional disturbance produced by the radium treatment. The patient went home after three weeks. The tumour had slightly decreased, and pain was much less marked. She came up for further treatment four months later, when surface applications only were used—over the right breast, axilla, above the clavicle, and on the left breast, which was involved in a lesser degree. Surface treatment on the first occasion could not be carried out in these areas, as she got as much radium treatment as she could stand at the time. The patient now, seven months later, writes to say that the right breast is practically normal in size and shape, that she feels well, but she has some burning in both breasts at night-

time, which she attributes to the radium treatment. It is almost certain that if radium had not been used in this case the right breast would have fungated through the skin some months ago.

10th to 12th September—B.D., 86·6 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R.* = 21·6 *mcd.* 6 *of* 1.*T.*5 × 4.*P.* = 24.*A.R.*, 65·0 *mcd.* 54 *hours. Total* = 48.*A.R.*

28th December, 1926, *to 6th January*, 1927—*S.D. Both breasts and sides of chest in both axillæ above both clavicles and on R. side neck.* 271·6 *mcd.*

B.—*Cancer of Uterus.* (9 *Cases.*)

Body (3 *Cases*). *Case* 1.—Aged 72. Had a pelvic tumour extending nearly up to the umbilicus, with ascites. The cervix was fixed, the top of the vagina was infiltrated, and a tube could not be introduced into the cancerous body by the gynæcologist. A tube, with the cells laid transversely, was therefore applied to the growth in the vagina, and a big surface dose given over the lower abdomen. The tumour decreased in size, the patient was more comfortable, on account of relief from pain, but died a month later from heart failure. Her heart condition had prevented her being operated on some months previously.

I.D., Vagina, 8·4 *mcd.* 2.*T.*26. 21 *hours. S.D., on abdomen and sacrum,* 143·3 *mcd.*

Case 2.—Was radiated after an unsuccessful attempt to remove the diseased body of the uterus on account of adhesions to the surrounding structure of the pelvis. She was treated by a tube in the uterus and surface radiation. Ten months later she had some recurrence in the operative scar of the abdomen, but is still able to be about.

I.D., Uterus, 23·7 *mcd.* 2.*T.*10 × 3.*P.* 17 *hours. S.D., above pubes,* 58·8 *mcd.* 45 *hours.*

Case 3.—Had her uterus removed in 1924. When treated in 1926, there was an extensive growth, involving 9 centimetres of the vagina. She was treated by tubes in the vagina and surface radiation, and when examined 11 months later no trace of disease could be found. Apparently cured.

I.D., Vagina, 36·0 *mcd.* 2.*T.*26 × 3.*R.* 4.*T.*15 × 1.*P.* 4.*A.R. S.D., Pubes,* 9·5 *mcd.*

Cervix (6 *Cases*). *Case* 1.—Had several very severe hæmorrhages, and when put on the table for radium treatment collapsed from loss of blood, and was apparently on the point of death. Tubes were applied to the vagina and cervix without an anæsthetic, but it was impossible at the time to do more manipulation. A fortnight later all hæmorrhage had ceased. The tumour at the top of the vagina was decreased in size, and it was possible to insert a tube to the fundus of the uterus. The patient was in a very weak condition for about three months, when she was able to return home. Improvement was slow, but continuous, for about six months afterwards, when she was lost sight of. Her death was reported fourteen months after radium treatment.

29th to 31st January—I.D., Vagina, 34·8 *mcd.* 16.*S.T. A.*4 × 7 *cm.*

13th to 14th February—I.D., Uterus, 27·9 *mcd.* 2.*T.*10 × 3.*P.* 30 *hours.*

Case 2.—This patient had an extensive excavation, replacing the cervix, and the growth extended down the anterior vaginal wall. She was treated by tubes in the cervix, and a small surface dose, and three months later the excavation of the cervix was smaller, with very little discharge, and the patient was much more comfortable. Two months later the condition appeared unchanged, after which she gradually lost ground, and died six months after radium treatment.

I.D., Cervix and vagina, 27·0 *mcd.* 2.*T.*10 × 3.*P.* 30 *hours. S.D., Pubes and sacrum,* 27·6 *mcd.*

Case 3 and *Case* 5 were lost sight of after treatment.

Case 4.—This patient received a prophylactic dose of radiation three weeks after total hysterectomy, nine months ago, and has remained well since.

I.D., Vagina, 8·0 *mcd.* 2.*T.*26.

Case 6.—This case had a cancerous growth the size of a golf ball at the top of the vagina six months after a Wertheim operation. It is known her condition improved for some time after treatment. Lost sight of.

B.D., Vault Vagina, 7·8 *mcd.* 6.*R.N. I.D., Vagina,* 9·4 *mcd. S.D., Perineum and abdomen,* 30·2 *mcd.*

C.—*Cancer of Mouth.* (23 *Cases.*)

Tongue (11 *Cases*). *Case* 1.—Aged 82. Had been operated on in October, 1925, for cancer of the tongue, and, as the growth had recurred, she was given radium treatment six months later as a palliative measure. This relieved the pain, to some extent, and lessened the amount of growth. She was given a small dose two months later, to relieve pain on a sore spot at the tip of the tongue. Treatment, which had to be limited, stayed the progress of the disease. She died in October, 1926, with chest trouble, from which she had always suffered, but had for some time been completely free from pain in her tongue. A point of interest in this case is that her hearing seemed to be greatly improved two or three months after radium treatment.

I.D., Mouth, 1·8 *mcd. Box,* 2 × 2 *cm.* 1⅔ *hours. S.D., under jaw and L. neck,* 24·4 *mcd.*

Case 2.—This patient was a frail old man of 67, who had considerable growth in the tongue and the interior part of the floor of the mouth. This improved after radium treatment, but the patient went down-hill, and died three months later.

S.D., under jaw and both sides of neck, 65·8 *mcd.*

Case 3.—Had an extensive growth of the tongue, with glandular involvement. His pain and discomfort were relieved when he left hospital for the country, but I understand he died some months later.

B.D., 12·3 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R. S.D.,* 43·2 *mcd.*

Case 4—Was treated in August for growth in his tongue, which was ulcerated opposite the fauces. After treatment, he was relieved of pain, and states that sometimes he forgot his tongue had ever been sore, and his general health was greatly improved. The induration of the tongue became more localised, and the ulcer diminished in size, being reduced to a clean slit, which had not a malignant appearance eight months after treatment.

B.D., 9·6 *mcd.* 6.*R.N.* × 3.*P.* = 18.*A.R. S.D.,* 62·4 *mcd.*

Case 5.—Had an extensive growth on the tongue, with a cavity underneath. He was treated in February and April, 1926, and has since been lost sight of.

Case 6.—Had his tongue removed in 1925, and was treated ten months later for recurrence on the floor of the mouth. Three months later his condition appeared to be very satisfactory, but he died six months after treatment. A fistula is reported to have developed through the floor of the mouth, accompanied by some hæmorrhage.

B.D., 15·7 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R. S.D.,* 38·1 *mcd.*

Case 7.—Had a recurrence in a small piece of the tongue, which had been left after an excision three and a half years previously. Twelve months after first treatment there was still a small nodule in the tongue and some induration under the jaw, with a certain amount of pain. The patient looks well, and the disease appears stationary.

4th to 8th March—B.D., 10·0 *mcd.* 6.*R.N.* × 2.*P.* = 12.*A.R. S.D.,* 60·2 *mcd.*

23rd to 29th November—B.D., 4·0 *mcd.* 6.*R.N. S.D.,* 48·8 *mcd.*

Case 8.—Had a small portion of his tongue removed in March, 1926. In September, when treated, the whole tongue was swollen, and very indurated. Induration extended to the floor of the mouth. He had had several hæmorrhages before treatment, and one severe one during treatment, but since then the hæmorrhages have completely stopped. He returned from the country, and was treated again in December, when the tongue was found to be considerably reduced in size. His general health had improved, and he was able to speak more distinctly.

26th to 29th September—B.D., 13·3 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R. S.D.*, 40·4 *mcd.*

20th to 23rd December—I.D., on dorsum and under tongue, 8·4 *mcd.* 10.*S.T. on dorsum tongue, and* 2 *of I.T.*5 *under each side of tongue. S.D., floor of mouth and both sides neck,* 84·3 *mcd.*

Case 9.—Had an indurated mass at the base of his tongue, with glands in the floor of the mouth and neck. He was treated in August, and when last seen there was considerable reduction in the induration of the tongue and the glands of the neck.

B.D., 12·0 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R.*. *S.D.*, 60·5 *mcd.*

Case 10.—Treated in the beginning of November, 1926; had a large, thickened mass in the anterior right half of his tongue, with an extensive ulcerated area beneath it. Speech hardly intelligible, and great pain. Patient looked very ill. When seen on the 22nd February, 1927, he states he "sometimes forgot his tongue," but has occasional neuralgic pains, which are relieved by aspirin. The surface under the tongue is clean, but a fissure-like ulcer remains. There is a small pimple near the tip of the dorsum of the tongue, with surrounding induration.

B.D., 10·4 *mcd.* 6.*R.N.* × 2.*P.* = 12.*A.R. S.D.*, 47·2 *mcd.*

Case 11.—There was a malignant mass in this patient's tongue opposite the left fauces, which was ulcerated. Treated December, 1925. Three months later there was a healthy scar at the site of disease; some thickening of tongue and floor of mouth. Looked well; could eat well. Condition apparently very satisfactory, and remained so for three months while under observation. Seen again December, 1926, when he had an indurated mass under the jaw and a growth on his palate. He was given surface radiation, and has not been seen since.

6th to 9th December—S.D., 62·1 *mcd.*

Palate (6 *Cases*). *Case* 1.—Small growth, possibly pre-cancerous, junction of hard and soft palate, no glands. Treated in January. Completely disappeared in a couple of months, and has remained well ever since.

I.D., 2·0 *mcd. F., Top of thermometer case.* 10 *minutes. F.*, 0·5 mm. *Brass.* 30 *minutes. F.*, 2·0 *mm. Brass.* 30 *minutes. d.*, 4 *mm.*

Case 2.—Was treated for a growth in the soft palate in May. When seen in September palate appeared normal, but for ten days there had been ulceration in the fauces, with slight induration in the tongue. He was again treated, and returned to the country, and has been lost sight of.

12th to 22nd May—B.D., Fauces and Palate, 4·8 *mcd.* 3.*R.N., Fauces,* 2·0 *mcd.* 3.*R.N., Palate,* 2·8 *mcd. S.D.*, 24·4 *mcd.*

8th to 11th November—B.D., Fauces and tongue, 7·5 *mcd.* 6.*R.N.* (3.*R.N. through floor of mouth*), *S.D.*, 31·3 *mcd.*

Case 3.—Had an extensive growth in his palate, which had perforated into the nose. Treatment in May cut down the malignant tissue, and relieved his symptoms of pain and discomfort. He was again treated in August, when there

was some recurrence. After temporary improvement, the disease slowly progressed, and he died seven months later.

15*th to* 17*th August—B.D.*, 4·0 *mcd.* 6.*R.N. S.D.*, 50·5 *mcd.*

Case 4.—Was treated for a growth on the palate and fauces in July. In October the growth was replaced by a small, healthy-looking ulcer, which, for the most part, had healed. Glands L. neck had disappeared; some persisted R. neck. A small lump in the anterior part of the tongue was healed. Patient's general condition had greatly improved. He returned to the country, and has been lost sight of.

5*th to* 6*th July—B.D.*, 8·4 *mcd.* 6.*R.N.* × 2.*P.* = 12.*A.R.*

13*th to* 18*th October—B.D., Tongue and floor mouth,* 16·8 *mcd.* 6.*R.N.* × 3.*P.* 18.*A.R. S.D.*, 63·4 *mcd.*

Case 5.—Was treated, two years after the removal of squamous cancer of the palate, for an enormous mass of glands under the right sterno-mastoid. Seven weeks later the mass was still hard, but somewhat smaller, and the pain less; general condition improved.

B.D., 9·6 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R. S.D.*, 38·0 *mcd.*

Case 6.—This patient had cancer of the palate, extending to the tonsil, and has not been seen since treatment in June last.

B.D., 7·2 *mcd.* 6.*R.N. S.D.*, 50·9 *mcd.*

Tonsil (2 *Cases*). *Case* 1.—Had a growth on both tonsils and the intervening palate, left tonsil being the worse. He was treated in January. He states he was very well till the following June, and when seen in the beginning of August the growth of the left tonsil had almost gone, while that on the right, which was originally the smaller growth, and received half the dose, had not improved to nearly the same extent after treatment. He returned to the country, and was lost sight of.

21*st to* 30*th January—B.D., left tonsil,* 6·9 *mcd.* 6.*R.N. R. tonsil,* 3·2 *mcd.* 6.*R.N. S.D., record lost.*

24*th August to* 2*nd September—B.D., right soft palate and fauces,* 5·0 *mcd.* 6.*R.N. S.D.*, 75·5 *mcd.*

Case 2.—This patient had a comparatively small malignant ulcer on the right tonsil, which completely disappeared after treatment, in December, 1925. He had some recurrence four months later, which did not respond well to treatment. He died from asthenia in August.

14*th to* 16*th December,* 1925—*B.D., Tonsil,* 1·8 *mcd.* 6.*R.N. S.D.*, 53·9 *mcd.*

Mucous Membrane (*Cheek*) (2 *Cases*). *Case* 1.—Had an extensive epithelioma inside her cheek, which did not respond well to radium treatment, which she received in November, 1925. She was again treated in February, 1926, and has since been lost sight of.

9*th to* 12*th November,* 1925—*B.D., through cheek to base of ulcer,* 4·9 *mcd.* 6.*R.N.* × 3.*P.* = 18. *A.R. S.D., Ulcer,* 6·0 *mcd. S.D., under jaw,* 14·0 *mcd.*

25*th January to* 2*nd February—I.D.*, 3·2 *mcd. Box, S.D.*, 36·4 *mcd.*

Case 2—Has not been heard of since treatment in August, when he returned to the country.

B.D., Cheek, 9·6 *mcd.* 6.*R.N.* × 3.*P.* 18.*A.R. S.D.*, 70·3 *mcd.*

Mucous Membrane (*Lower Alveolus*) (2 *Cases*). *Case* 1—Had a very extensive growth on the floor of the mouth, and was only treated by surface application. He died about ten weeks later from septic absorption.

Case 2.—This patient was similar to the former one, and was given surface treatment alone in May. A sinus occurred, and he was given a further treat-

ment in August; since then he has been lost sight of. Before leaving hospital all his pain had gone, and he was gratified with the relief obtained.

Cancer of Pharynx. (3 *Cases.*)

Case 1.—This case was lost sight of.

I.D., 3·3 *mcd.* 2.*T.*10. 2 *hours. S.D.,* 29·5 *mcd.*

Case 2.—Had a post-cricoid carcinoma involving the œsophagus, through which a tube was inserted by the throat specialist, using direct vision. The patient did very well, and is now, ten months afterwards, apparently free from disease. Microscopically, the tumour was squamous carcinoma. I considered this patient had some chance of doing well at the time of treatment, as I could thoroughly radiate the growth from inside and by surface application. Apparently cured, according to specialist's report.

I.D., Œsophagus and Pharynx, 5·5 *mcd.* 2.*T.*8. × 3.*P.* 4¾ *hours.*

S.D., across neck area of growth, 38·0 *mcd. A.A.*2 × 15 *cm.* 33 *hours.*

Case 3.—Had an extensive extrinsic carcinoma of the larynx and lower pharynx, and was only able to swallow liquids with difficulty. It was found impossible to insert a tube through the growth by the throat specialist. She was only given surface radiation. She was rapidly going down-hill on account of inability to swallow and take sufficient nourishment. I performed a gastrostomy under local anæsthetic three weeks after radiation. Immediately after radiation there was some improved ability to swallow, and seven weeks after radiation she was able to take bread by the mouth, which she did of her own accord, in spite of having a gastrostomy opening. Four months after radiation she was apparently doing well.

S.D., 38·4 *mcd.*

Cancer of Œsophagus. (2 *Cases.*)

Case 1.—Had a growth in the œsophagus 34 cm. from the lips, through which a tube was inserted by the throat specialist with the aid of an œsophagoscope. Case has been lost sight of.

I.D., in œsophagus, 8·4 *mcd.* 1.*T.*5 × 3.*P.*

Case 2.—Had a growth at the lower end of the œsophagus, through which it was found impossible to get a tube. She was given surface radiation opposite the stricture, after which she was able to swallow better, and felt more comfortable; but the treatment was purely palliative, and the patient died two and a half months later.

S.D., back and front trunk, 64·4 *mcd.*

Cancer of Stomach. (1 *Case.*)

Had a palpable tumour of the pylorus, and was in great pain and discomfort. Unable to take food, vomited frequently, and was suffering from loss of sleep. Three months before treatment her condition was considered inoperable. After radiation, her symptoms gradually improved. Two and a half months after treatment she had no pain, no vomiting, the lump had become smaller, but there was considerable ascites, and the patient was slowly becoming weaker. She died four months after radiation.

S.D., front and back trunk, 125·1 *mcd.*

Cancer of Rectum. (3 *Cases.*)

Case 1 and *Case* 2 were given fairly extensive treatment by radium tubes in the rectum, but were lost sight of immediately after treatment.

Case 1.—*I.D., Rectum,* 28·0 *mcd.* 2.*T.*15 × 5.*P. S.D.,* 25·3 *mcd.*

Case 2.—*I.D.,* 24·0 *mcd.* 4.*T.*15 × 3.*P. S.D.,* 14·0 *mcd.*

Case 3.—Had his rectum removed in 1922 (October) for cancer, and was treated for some local recurrence in July, 1923, which cleared up after radiation. There was recurrence in the neighbourhood of the right sacro-iliac joint, accompanied by great pain, for which he was treated in December, 1926. His condition improved, but he succumbed to an intercurrent attack of influenza.

5*th to* 7*th July,* 1923—*I.D., Sinus site of rectum,* 5·3 *mcd.* 2.*T.*7 × 2.*P. S.D.,* 27·6 *mcd.*

17*th to* 20*th December,* 1926—*S.D., back of pelvis,* 99·8 *mcd.*

Cancer of Orbit. (1 *Case.*)

This patient had a growth in the left lachrymal gland, and I am indebted to Dr. Euphan Maxwell for her notes on this case, especially with reference to the effect of radiation on the retina. In 1922 the left eyeball had 5 mms. horizontal proptosis, the vision was 6/6ths with glasses, and the patient was given 11 X-ray exposures. In February, 1926, the tumour and gland had markedly increased, with great pain and diplopia, horizontal proptosis 6 mms., vision 6/18ths with glasses, retinal vessels congested, pigmentary disturbances in macular area, the result of posterior pressure. First treatment with radium was in April, 1926, when a comparatively small dose was given, on account of fear of injury to retina. In June, 1926, tumour appeared softer, pain was considerably less, horizontal proptosis increased to 11 mms., vision *in statu quo,* In September, 1926, a second treatment with radium was given. As the case now appeared desperate the effect of radiation on the retina was disregarded, and nearly three times the original dose was given by needles inserted into the tumour in a row about a centimetre above the upper part of the retina. In December, 1926, the horizontal proptosis was reduced to 6 mms., congestion of retinal vessels disappeared, vision 6/9ths with glasses. In February, 1927, proptosis was 7 mms., vision *in statu quo,* no diplopia or pain. During the intervals of treatment patient has continued her work.

28*th to* 30*th May—B.D.,* 3·2 *mcd.* 6.*R.N. S.D., Forehead and temple,* 12·5 *mcd.*

18*th to* 19*th September*—8·0 *mcd.* 6.*R.N.* × 2.*P.* = 12.*A.R. S.D., Forehead and temple,* 9·6 *mcd.*

Cancer of Penis. (1 *Case.*)

This patient was treated for horny growths on the glans and scabs, which had persisted for eighteen months after amputation of prepuce. These disappeared a month after radiation, leaving an ulcer in one place, which appeared malignant. Patient has not been seen by me since.

S.D., Glans Penis, 10·2 *mcd. Glands Groin,* 19·0 *mcd.*

Cancer of Lip. (4 *Cases.*)

Case 1.—Appeared to be cured when examined three months after radium treatment.

S.D.—Record lost.

Case 2.—Was a very advanced case, in which there was an opening through the floor of the mouth, and treatment had to be interrupted, as the patient's

condition was so serious from septic pneumonia, to which he succumbed a few days later.

S.D., 24·0 *mcd.*

Case 3.—Was treated ten months after extensive operation for removal of cancer of the lip and glands, which had recurred, and the original incision in the middle line was breaking down. His condition was stated to be much improved four months after radiation.

B.D., floor of mouth, 12·0 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R. S.D.*, 52·9 *mcd.*

Case 4.—Treated two months after an extensive operation in the lip, which had not healed. Two months later the operation scar had healed, and the patient looked much better, and the moderate amount of pyorrhœa from which he had originally suffered, and for which he was given an additional small amount of radiation in the mouth, had disappeared.

S.D., Lip and floor of mouth, 61·1 *mcd. I.D., Mouth, opposite operation scar,* 1·6 *mcd.* 2.*T.*10. 1 *hour. Pyorrhœa,* 1·3 *mcd.* 2.*T.*10 × 4.*P.* 50 *minutes.*

Cancer of Skin. (11 *Cases.*)

Case 1.—Had an extensive growth, involving the anterior half of the scalp, for which part of the frontal bone had been removed by operation some months previously. There was considerable tumour formation at the back of the malignant area. Four and a half months after treatment the tumour had disappeared. In front of this there was a raw area, with some malignant-looking thickening, and the anterior part of the diseased area had skinned over.

B.D., Anterior part of scalp, 9·0 *mcd.* 6. *R.N.* × 2.*P.* = 12.*A.R. B.D., Posterior part of scalp,* 14·0 *mcd.* 6 *of* 1.*T.*4 = 6.*A.R. S.D., Over ulcerated area* (1), 1·5 *mcd. Beta radiation d.* 1·5 *mm.*; (2) 21·0 *mcd.*; 2.*T.*10 *d.* 60 *mm. Total S.D.*, 22·5 *mcd.*

Case 2.—Had a fungating mass covering his right temporal region. This was scraped away 28 hours after radium needles were removed. Although the growth had originally been very hæmorrhagic, there was comparatively little bleeding during the operation. Two small arteries were tied. Four months after treatment the ulcer was reported to be still healing, and healthy in appearance.

B.D., Growth temple, 15·4 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R. Growth scraped away, and S.D.*, 15·4 *mcd.*

Case 3.—A tumour in the temporal region was removed from this patient, and radium box applied immediately after the operation, and left in position for five days. On the seventh day the incision was soundly healed. Usually with a dose such as this patient received, healing is delayed, and the scar stretches. Possibly the fact that this tumour turned out histologically to be squamous cancer enabled the skin in the area to stand a big dose without suffering, as, in my experience, it would have done, especially if the same dose had been given, using a large quantity of radium for a short period.

S.D., 2·56 *mcd. Box* 3 × 2 *cm. d.* 5 *mm.* 5 *days,* 1 *hour.*

Case 4.—A growth of lower eyelid was lost sight of soon after treatment.

B.D., 2·0 *mcd.* 6.*R.N. S.D.*, 0·5 *mcd.*

Case 5.—Was apparently cured after one radiation of tumour on the cheek.

S.D. (1), 1·2 *mcd.* 6.*R.N. on surface. A.A.*2 × 1·2 *cm., d.* 3 *mm.* (2), 0·8 *mcd., F.* 1·5 *mm., Pb., d.* 5 *mm. Total, S.D.*, 2·0 *mcd.*

Case 6.—Apparently cured after one treatment. No scar or mark visible on the nose at site of growth except slight smoothness of the skin, which is hard to detect.

S.D. (1), 0·4 *mcd. R. cells. A.A.*, 1·5 × 0·75 *cm. d.* 2·5 *mm.* (*beta rays*). (2), 1·2 *mcd. F.*, 1·5 *Pb., d.* 8 *mm. Total, S.D.*, 1·6 *mcd.*

Case 7.—Microscopically, squamous cancer of the skin on the nose was apparently cured after one radiation. Slightly depressed, inconspicuous white scar marks site of growth.

B.D., 0·7 *mcd.* *Area of growth*, 2 × 1·5. *S.D.* (1), 0·1 *mcd.* 6.*R.N.*, *d.* 1 *mm.* (2), 0·9 *mcd.* *F.*, 1·5 *Pb.*, *d.* 10 *mm.* *Total*, 1·7 *mcd.*

Case 8.—Growth on nose apparently cured after one radiation.

S.D. (1), 1·2 *mcd.* 6.*R.N.* *A.A.* 2·5 × 2·5 *cm.* (2), 3·2 *mcd.* *F.*, 1·5 *Pb.*, *d.* 10 *mm.* *Total* 4·4 *mcd.*

Case 9.—Had an extensive epithelioma of the cheek, which was so deep that it practically involved the mucous membrane. It was very much improved two months later. The patient was advised to return for further treatment, as it was inadvisable to attempt too much at one sitting, but failed to do so; and five months later ulceration was reported to be extending.

B.D., 10·4 *mcd.* 6.*R.N.* × 3.*P.* = 18.*A.R.* *S.D.*, *over growth*, 10·9 *mcd.*

Case 10.—Broken-down growth on chin, healed one month after radiation. Six months after radiation inconspicuous white scar remained, without any signs of induration or disease. Apparently cured.

S.D., 2·55 *mcd.* 4.*T.*15 *against growth.* 2¼ *hours.*

Case 11.—Was recently treated for a growth on back of hand. Is probably cured, judging by appearance when last seen.

S.D., 1·54 *mcd.* 10.*S.T.* *A.A.* 2 × 5 *cm. against skin.* 140 *hours.*

Sarcoma. (12 *Cases.*)

Intracranial (3 *Cases*). *Case* 1.—A decompression was performed on this patient on the 1st May for a tumour in the left occipital region, a piece of bone 13 cm. in diameter being removed. Extensive bulging of the brain and considerable hæmorrhage occurred at the operation. The case was diagnosed as an infiltrating glioma, with which it was impossible to deal by ordinary surgical methods. As the surgeon was anxious to remove the tumour, if possible, I arranged to give what I considered to be a necrotic dose in the tumour area, with the idea that the tumour area should subsequently be removed, and that the doubtful area at the periphery should get such a dose as would efficiently deal with the remaining malignant cells. The complication of hæmorrhage, I pointed out, was not likely to arise after the dose proposed. On the 18th May, three days after radiation, the central mass of the growth, which could now be differentiated from the rest of the brain by touch, owing to its tougher consistence, was removed. The roof of the lateral ventricle could be seen at the bottom of the cavity left by the tumour, which measured 4·5 × 4·5 × 3·5 cm. Very little hæmorrhage occurred during the operation, and the patient made a practically uninterrupted recovery from the immediate effects of the operation and radiation, and returned home. On the 7th August a cystic tumour recurred in the area. The patient was again operated on, and 2 or 3 small egg-shaped masses were removed, which were found microscopically to consist of inflammatory tissue only. The patient remained well, and was able to get about till the end of the year, when she again commenced to get headaches, and a swelling, obviously containing fluid, recurred in the region of the decompression. This had to be tapped twice a day to relieve the symptoms, and 10 to 40 ccs. were drawn off each time. I was again asked to radiate her, with the object of destroying the cells which were responsible for the secretion. She was treated on 13th January, 1927, after which the amount of fluid secreted diminished from day to day for about ten days, when it completely ceased, and the patient returned home.

13th to 15th May—*B.D.*, *Tumour brain*, 14·8 *mcd.* 6.*R.N.* × 3.*P.* = 18.*A.R.*

3.*R.N. inserted to depth of* 10 *cm.*, 3.*R.N. to* 8 *cm. Area of brain occupied by needles,* 4·5 × 3 *cm.* (*surface*) × 10 *cm.* (*depth*), *and S.D.,* 15·5 *mcd.*

13*th to* 15*th January,* 1927—*B.D., Cystic tumour brain,* 15·7 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R. S.D.,* 15·0 *mcd.*

Case 2.—About a week before radiation this patient had an extensive decompression performed in the left parieto-occipital region, but no definite tumour could be located. On this account the whole skull was given surface radiation, with cross-fire, without any apparent effect. The patient died about a month later, and at the post-mortem it was found that most of the tumour was under the area of decompression, but that it extended across the middle line, and there was considerable involvement on the right side. The case was quite hopeless from the beginning.

S.D., on skull, 101·7 *mcd.*

Case 3.—Was treated shortly after a decompression operation in front of the upper end of the Rolandic area. The tumour, which was apparently a sarcoma from the dura mater, could not be removed. He suffered from frequent fits, and had one during treatment, but had no fit for three weeks after leaving hospital. He returned for treatment five months later, as he was getting milder fits about once a week; there was marked pulsation in the area of decompression, but no projecting tumour as before. Three months later he was reported to be fairly well, but has not returned for treatment, as recommended.

1*st to* 4*th June—B.D.,* 5·6 *mcd.* 6.*R.N., into area.* 4 × 5 × 3 *cm. S.D.,* 33·3 *mcd.*

10*th to* 12*th November—S.D.,* 55·9 *mcd.*

Maxillary Antrum (1 *Case*).—Returned to the country after radiation for an extensive tumour in the maxillary antrum. His condition was considerably improved for some months after treatment, but he was recently reported to be disimproving.

B.D., through cheek, 9·9 *mcd.* 6.*R.N.* × 3.*P.* = 18.*A.R. I.D. R. nostril,* 11·1 *mcd.* 7.*T.*8 × 2.*P. S.D.,* 35·8 *mcd.*

Tongue (1 *Case*).—Was treated after operation with surface radiation alone, and disimproved very rapidly after leaving hospital. The tumour in the tongue, which had been easily shelled out, apparently was a rapidly growing sarcoma, and not the benign condition, for which he was treated. He died five weeks later with evidence of metastases.

Mucous Membrane, Lower Alveolus (1 *Case*).—Recently had had his arm amputated through the shoulder for malignant disease. He was treated for a tumour in the mouth, and suddenly collapsed a fortnight later. His condition was hopeless when first seen.

B.D., 14·2 *mcd.* 6.*R.N.* × 3.*P.* = 18.*A.R. S.D.,* 60 *mcd.*

Pharynx (1 *Case*).—Had an extensive growth, which blocked the upper end of the œsophagus, and glands both sides of neck. Immediate effect. Could swallow liquids without pain, and speak more distinctly. A fortnight after treatment she was able to eat a rasher. She died about a month after treatment.

I.D., Œsophagus and Pharynx, 9·0 *mcd.* 2.*T.*7 *and* 2.*T.*8 × 2.*P.* = 4.*A.R. S.D., from mandible to clavicle each side of neck and over pharynx,* 58·0 *mcd.*

Neck (2 *Cases*). *Case* 1.—Improved after treatment, but was lost sight of.

Case 2.—Considerably improved after first treatment, in June, 1926. He was again treated in November, in an attempt further to reduce hard glandular tumours on both sides of neck. He died about a month later with symptoms of toxæmia, following erysipelas.

27th October to 2nd November—S.D., 97·8 *mcd.*

Uterus (1 *Case*).—Was not considered suitable for operation, and has apparently made a complete recovery after radiation, according to report received 13 months after treatment from the gynæcologist in charge.

I.D., Uterus and cervix, 28·0 *mcd.* 2.*T.*10 × 3.*P.* 43 *hours.*

Vulva (1 *Case*).—Had received radium treatment about three months before she came under my care at Steevens'. There was extensive growth on both sides of the vulva, which appeared to be increasing. After treatment, she felt much more comfortable, and returned home. She was re-admitted to hospital two months later, and on examination the right side, to which the growth had extended, was apparently well, while there was a deep ulcer on the left side, with smooth edges and fairly clean base, with very considerable induration round it. It is sometimes extremely difficult to decide whether such a condition is due to efficient radiation, and is a manifestation of prolonged reaction, or whether the process is malignant. At this time the patient had considerable discomfort and pain. I decided not to give her any more radium. The condition improved with aseptic dressings and the radiant heat bath, and she was able to return home with the ulcer practically healed. Six months later, I hear that she is still doing well.

B.D., Vulva, 10·8 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R. S.D., Perineum,* 22·6 *mcd.*

Humerus (1 *Case*).—Was treated in January for an extensive growth, which involved the upper half of the diaphysis of his humerus. The tumour in this region disappeared, and the arm became very much thinner than his normal one. In spite of the local disappearance of the tumour, the X-ray appearances of the bone on several occasions did not materially alter following the treatment. About two months after treatment he had a very considerable pain in the arm, probably due to the effect of radiation on the nerves. This lasted for about a month, and then completely left him. About August he commenced to lose ground, and the tumour then spread to the axilla and pectoral region, in which area he was treated in October, but he rapidly went down-hill, and died about a fortnight later.

16th to 21st January—S.D., Arm and shoulder, 166·6 *mcd.*

2nd to 4th February—B.D., Arm, 17·2 *mcd.* 6.*R.N.* × 4.*P.* = 24.*A.R.*, 62·0 *mcd.*, 6 *of* 1.*T.*4 × 4.*P.* = 24.*A.R.* 43 *hours. Total,* 79·2 *mcd.*, 48.*A.R.* (*One tube went through the shaft of the bone.*)

Rodent Ulcer. (8 *Cases.*)

Case 1.—An extensive rodent ulcer of the ear has not been seen since treated in April last.

S.D., 2·4 *mcd.* 2.*T.*7, 2.*T.*8, 2.*T.*10 *against ulcer.* 1 *hour* 40 *minutes.*

Case 2.—Also of the ear; has not been seen since treatment.

S.D., 4·5 *mcd.* 2 *boxes of* 3 × 2 *cm.; one in front, one behind ear. d.* 5 *mm.* 7 *hours.*

Case 3.—Involving the outer canthus of the right eye and neighbouring conjunction. Has been under treatment since 1924. Although there is no ulceration there at present, there is a nodule at the outer canthus, which is suspicious, and will require further treatment, and will probably be best dealt with by the insertion of one or two serum needles containing glass emanation capillaries.

I.D., under eyelid, 0·3 *mcd.* 1.*T.*5. *S.D.,* 3·3 *mcd. Box* 3 × 2 *cm. d.* 10. *mm.* 5 *hours.*

Case 4.—Rodent ulcer of the eyelid; apparently cured after one treatment.

S.D., 0·7 *mcd.* 6.*R. cells. A.A.,* 1·5 × 0·75 *cm. against ulcer.* 1·4 *mcd.* + *F.,* 1·5 *mm.* − *Pb., d.* 12 *mm.*

Case 5.—The contents of this country patient's orbit were destroyed by a long standing rodent ulcer. He has not been heard of since treatment.

S.D., (1), 2·8 *mcd.* 2.*B. of* 3 × 2 *cm. d.* 3·5 *mm.* (2), 25·7 *mcd. A.A.* 5 × 7 *cm. d.* 20 *mm.* (3), 8·1 *mcd. d.* 50 *mm. Total,* 36·6 *mcd.*

Case 6.—With a rodent ulcer of the nose; was apparently cured after three treatments—in September and October, 1925, and January, 1926.

13*th September,* 1925—*S.D.,* 4·0 *mcd. Box* 3 × 2 *cm. d.* 6 *mm.* 5 *hours.*

31*st October,* 1925—*S.D.,* 1·3 *mcd.* 2.*T.*7, 2.*T.*8 *against ulcer.* 5½ *hours.*

28*th January,* 1926—*S.D.,* 2·9 *mcd.* 2.*T.*15 × 2.*P. against ulcer.* 3⅓ *hours.*

Case 7.—With rodent ulcer of cheek; was apparently cured after one treatment.

S.D., 3·2 *mcd. Box* 3 × 2 *cm. d.* 5 *mm.*

Case 8.—With rodent ulcer of cheek; was apparently cured after one treatment.

S.D. (1), 0·2 *mcd.* 6.*R. cells. A.A.* 1·5 × 1·3 *cm. d.* 2 *mm.* (2), 0·7 *mcd. F.* 2·5 *mm. Brass. d.* 6 *mm.* (3), 2·6 *mcd. d.* 20 *mm. Total,* 3·5 *mcd.* 8¾ *hours.*

Non-Malignant Conditions. (100 Cases.)

F.—*Fibrosis Uteri.* (1 *Case.*)

Was having excessive hæmorrhages before treatment; has had only one hæmorrhage, which was rather severe, since treated in June last, and has remained quite well ever since.

I.D., Cervix, 12·7 *mcd.* 3.*T.*10. 21¾ *hours. S.D., Pubes,* 10·9 *mcd.*

Abdominal Adhesions. (3 *Cases.*)

Case 1.—Post-dysenteric adhesions. Had for years been suffering from pain and a tender spot to the left of the umbilicus, which was considered to be caused by an adhesion. For a couple of months after treatment there was apparently no great difference, but since then the patient has remained perfectly free from any discomfort.

S.D., 30·3 *mcd.* 44 *hours.*

Case 2.—Appendicectomy. Post-operative adhesions. Has not up to the present had any material benefit from the treatment.

S.D., 45·2 *mcd.* 19¼ *hours.*

Case 3.—Had considerable relief of pain and tenderness in the scar area, which was very extensive, following an emergency operation for ruptured gastric ulcer.

Actinomycosis. (1 *Case.*)

Came for treatment with a diagnosis of inoperable malignant disease; had an indurated swelling in his neck, and a number of glands. After radiation the tumour broke down fairly rapidly, and pus, containing greenish, seed-like bodies, had to be evacuated on several occasions. The whole condition cleared up in about six weeks.

S.D., 42·8 *mcd.* 72 *hours.*

Addison's Disease. (2 *Cases.*)

Case 1.—Was in an advanced stage of the disease when, at the request of the physician in charge, she was radiated in the hope that it might help her, as it had done a tuberculous peritonitis case, who had recovered two years before. She had suffered from vomiting, extreme loss of appetite, weakness, general discomfort, and pains. Her blood pressure was 60. Shortly after radiation she was able to take more nourishment, and asked for food; was relieved of pain, appeared physically stronger and more alert, ceased vomiting, and her blood pressure at one time rose to 90, but for a considerable time was 80. On the 86th day after treatment commenced she unexpectedly collapsed, and died in a few hours, at a time when the rest of the household had colds. There is no doubt that her symptoms were alleviated, and for a time she appeared distinctly to be improved.

S.D., front and back trunk, supra-renal area and spleen.

15*th to* 16*th September,* 17·5 *mcd.* 18 *hours.* 21*st September to* 23*rd October,* 18·2 *mcd.* 23*rd October to* 2*nd November,* 3·4 *mcd.* 6*th November to* 4*th December,* 30·3 *mcd.* 4*th December to* 10*th December,* 10·4 *mcd. Total,* 79·8 *mcd. during* 78 *days of* 86*-day period.*

Case 2.—Appetite was improved for a short time after radiation, but the benefit derived from the treatment was not marked, as in the first case. As he was unwilling to make a prolonged stay in hospital, the dose was modified, and he only received one exposure, and no prolonged weak radiation, such as experience has shown to be the most beneficial in grave tuberculous conditions.

S.D., front and back trunk, supra-renal area, and spleen, 17·4 *mcd.* 68 *hours.*

Adenitis. (2 *Cases.*)

Case 1.—Aged 5 years. Glands after scarlatina. *Case* 2.—Aged 19 years. Glands, accompanying tonsilitis. In both these cases the disappearance of glands appeared to be hastened by radiation.

Case 1, *S.D.,* 2·3 *mcd.* 16 *hours. Case* 2, *S.D.,* 15·6 *mcd.* 23 *hours.*

Corns. (1 *Case.*)

One corn disappeared temporarily in this case, but recurred, as the cause was not removed.

S.D., against corn, 1·6 *mcd. Box* 2 × 2 *cm. F.* 2 *mm. brass.* 1½ *hours.*

Deafness. (15 *Cases.*)

The clinical details of most of these cases, for which my colleague, Mr. T. G. Wilson, is responsible, have been published in a separate paper. It is too early to say whether the improvements obtained are permanent or not; but if it can be proved that radiation stays the course of some forms of deafness,

which are usually progressive, it will be of great help in the treatment of this distressing disability. The dosage used up to the present has been very small.

I.D., in external ear, 0·15 *to* 0·5 *mcd. S.T., containing* 1·0 *to* 4·0 *millicuries, used for* 24 *hours, inner end of agent* (*radium emanation*), 4 *mm. from tympanum.*

Dental. (1 *Case.*)

This patient was treated after a dental clearance of all but four lower incisor teeth. The result appears to have been that the process of healing was very materially hastened, and an artificial denture could be worn with comfort a fortnight after extraction. Similar rapid healing has been noticed when septic teeth have been removed during radium treatment of oral cancer.

I.D., in mouth, 0·5 *mcd. Beta radiation.* 6.*R.N. in thin test tube. Various positions in mouth.*

Erysipelas. (1 *Case.*)

Radiation was used in this case as a tentative measure to see if it would help. The treatment certainly did no harm; and, judging by the effect of mild radiation on inflammatory conditions, it would appear to be a rational procedure.

S.D., 3.4 *mcd.* 41 *hours. Area,* 192 *sq. cms.*

Gangrene. (2 *Cases.*)

Case 1.—Had to have her arm amputated following septic tonsilitis. Radium was applied to the area of the occluded subclavical artery after the fingers had become black and shrivelled, with the idea of improving the nutrition of the limb. The line of demarcation appeared to form more quickly than was expected.

S.D., above and below clavicle, 21·6 *mcd.* 31 *hours.*

Case 2.—Senile gangrene, starting with an ulcer on the big toe in May. Nine days' radiation of the whole foot, in July, was followed by the disappearance of pain, swelling, and discolouration, increased pus formation, followed by repair of ulcer till nearly healed. Four months later the ulcer commenced to break down, and went from bad to worse, till the whole toe became gangrenous in February, 1927. Subsequent radiation of the femoral artery had no obvious effect. No line of demarcation has yet been formed.

1*st to* 9*th July—S.D., ulcer and whole foot,* 13·2 *mcd.* 9 *days.*

Injury. (3 *Cases.*)

Case 1.—Painful knee after fracture of femur. Pain relieved and movement increased after radiation.

S.D., round knee, 14·1 *mcd.* 24 *hours.*

Case 2.—Old fracture of wrist. Function of hand improved.

S.D., Wrist and forearm, 38·1 *mcd.* 68 *hours.*

Case 3.—Old injury to knee. No appreciable improvement in pain or function.

S.D., round knee, 54·8 *mcd.* 5 *days* 19 *hours.*

Keloid. (3 *Cases.*)

Cases 1 and 2 not seen since treated.

Case 3.—Great improvement in function of hand, and disappearance of keloid tissue resulted.

S.D., back of hand, 12·7 *mcd. A.A.* 5 × 7 *cms.*

Naevus. (3 *Cases.*)

Case 1.—An infant. Had been suppurating for five months after the application of carbon dioxide snow to the centre of the naevus on the cheek and temple, and the ulceration would not yield to any form of treatment. Three weeks after mild radiation this septic condition completely cleared up, leaving a smooth, healthy skin surface. The patient is still under treatment for the extensive areas of naevoid tissue which remain.

S.D., Cheek and temple, 1·2 *mcd. A.A.* 2 × 8 *cm. d.* 3 *mm.* 5 *hours.*

Case 2.—Had a small naevus on the nose, which frequently bled. It bled once immediately after treatment, but has not done so now for five months. The original papule at one time completely disappeared, but has recurred in a modified form.

S.D., 0·14 *mcd. Beta radiation.* 15 *minutes.* 76·3 *mcs. in capillaries in* 1·0 *mm. glass tube. d.* 3 *mm.*

Case 3.—An infant, with an ulcerating naevus on the abdomen; was healing when last seen, but was lost sight of.

S.D., 6·0 *mcd. A.A.* 4 × 7 *cm. d.* 7 *mm.* 7 *hours.*

Papilloma. (2 *Cases.*)

Case 1.—Papilloma of the tongue; cured after one treatment with combined beta and gamma rays. Tongue sore for a week, ten days after treatment.

I.D., beta rays, 0·057 *mcd.* 3 *minutes.* 153 *mcs. in capillaries. d.* = 5 *mm. I.D., gamma rays,* 0·38 *mcd. F.* 1·75 *mm. brass.* 20 *minutes. d.* = 5 *mm.*

Case 2.—Papilloma of the palate cured after two beta ray treatments. Warty growth fell off twelve days after second treatment; small ulcer, 18th day. Healed two weeks later.

28*th September—I.D.,* 0·14 *mcd.* 4 *minutes.* 190 *mcs. in capillaries in* 2.*T.*15 *open at end. d.* 4 *mm.*

9*th November—I.D.,* 0·18 *mcd.* 10 *minutes.* 140 *mcs. in capillaries in* 2.*T.*10 *open at end. d.* 1·3 *mm.*

Pleurisy. (1 *Case.*)

Immediately after radium was removed the breath sounds in the patient, according to the physician's report, could be more distinctly heard than before radiation. Patient felt better, and has been lost sight of since discharged from hospital a couple of days later.

S.D., 46·9 *mcd.* 6 *days.*

Prolapsed Rectum. (1 *Case.*)

Refused operation for prolapse of long standing, and radium, which was available, was applied to investigate its effect. The size of the swelling was very much reduced a few days after radiation; and, as there had been no change during six weeks in bed before that, this treatment would appear to have had some effect. Ice bags and mechanical pressure had not improved the condition.

S.D., 13·2 *mcd. A.A.* 6 × 2 *cm.* 18 *hours.*

Pruritus. (2 *Cases.*)

Case 1.—Of the anus; was apparently cured after one treatment, but complete relief did not take place for about six weeks.

S.D., 4·6 *mcd.* 16.*S.T. A.A.* 2 × 8 *cm. against skin.* 9 *hours. d.* 10 *mm. wool.*

Case 2.—Of the vulva and anus; cured as in Case 1.

S.D., 12·1 *mcd.* 44.*S.T.* *A.A.* 2 × 22 *cm.* 18 *hours.* *d.* 2 *mm.* 14 *hours.* *d.* 15 *mm. wool.*

Rheumatism. (3 *Cases.*)

Case 1.—As on a previous occasion, patient was relieved of pain in the right shoulder after radium treatment. At the present time, eleven months later, this shoulder is well, but left shoulder is causing trouble.

S.D., 58·6 *mcd.* *A.A.* 2 × 6 *cm.* × 14.*P.* 14 *days.*

Case 2.—Small dose administered to ankle, giving some relief of pain, and diminished swelling. Even small doses had considerable constitutional effect on this patient, and made her feel sick and vomit on this and on previous occasions.

S.D., round ankle, 10 *mcd.* 4 *days.*

Case 3.—Rheumatism of the thumb of comparatively recent origin and lumbago of many years' standing were treated. The improvement in the thumb was not marked, but apparently there was a profound effect on the lumbago, and the patient was much more free from pain in the area treated during this winter than he has been for years. It is interesting that there was no change in adjacent untreated areas. A similar effect has been noticed in other cases in previous years.

S.D., Thumb, 6·7 *mcd.* 43 *hours.* *S.D., Lumbar region,* 44·9 *mcd.* 34 *hours.*

Scars. (17 *Cases.*)

Gunshot Wound Scars (11 *Cases*).—These are cases of army pensioners who have been under treatment from time to time for disabling wounds received during the Great War, and were attending the Special Surgical Clinic for re-educational exercises, massage, &c. Radium treatment is only considered when the above, or operative, treatment has ceased to improve the function of a limb. The disability is usually due to painful or adherent scars. Immediately after radiation they return to the massage department, and continue treatment until they are cured, or unlikely to further improve. Pain and tenderness are usually relieved almost at once, but the stretching of the fibrous tissue and the re-education of the pensioner to use his limbs properly takes some time.

Case 1.—Intense pain in stump of amputated finger completely removed, but some tenderness persisted.

S.D., 18·5 *mcd.* *A.A.* 12 × 10 *cm.* *d.* 20 *mm.*

Case 2.—Also a finger stump. Slight improvement. Neuroma subsequently removed by operation.

S.D., 18·5 *mcd.*

Case 3.—Symptoms not so severe as Case 1. Returned to work.

S.D., 11·4 *mcd.*

Case 4.—Very extensive scarring of forearm treated with large dose of cross-fire. As was expected, skin became inflamed, and was sore from the third to seventh week after treatment, which resulted in improved circulation of the hand, healing of long-standing trophic ulcers in the fingers, and increased power in extending the fingers.

S.D., 91·2 *mcd.*

Case 5.—Scars on forearm. Transferred to hospital, and lost sight of.

S.D., 18·6 *mcd.*

Case 6.—Scars on forearm. Grip of hand improved steadily from 20° to 80° (right hand 90°). Able to return to work.

S.D., 40·7 *mcd.*

Case 7.—Scars on wrist. Circulation of hand improved, and grip increased from 12° to 50°. Returned to work.

S.D., 33·4 *mcd.*

Case 8.—Scar on back of hand. Movements of hand freer, scar more supple, and less pain.

S.D., 39·0 *mcd.*

Case 9.—Scars on thigh. Sciatic pain relieved. Tremors of muscles disappeared. Movements of knee and ankle became almost normal. Patient states he was able to sleep at night. Condition on the whole was much improved, but has still some spasms of muscles.

S.D., 71·1 *mcd.*

Case 10.—Scar of leg adherent to tendo-Achillis. Functional movement of ankle improved. Scar less adherent. Pain relieved.

S.D., 20·9 *mcd.*

Case 11.—Scars of foot. Much bony deformity, due to bullet wound; foot very swollen, with some bone disease, and numerous sinuses. Movements of ankle practically nil. After radiation and rest in bed in hospital all the swelling in the soft tissues disappeared, the sinuses are practically healed, and movements of ankle are nearly full, only being restricted by bony deformity.

10*th to* 17*th June—S.D.*, 31·7 *mcd.* 7 *days.*

6*th to* 11*th September—S.D.*, 13·7 *mcd.*

Scars—Post-operative.—Two cases were given a small dose immediately after operation on the face to stimulate healing, with an inconspicuous scar, and one case after amputation of injured septic fingers, to hasten healing. Results satisfactory.

Case 1.—*S.D., Face,* 0·5 *mcd.* *Case* 2.—*S.D., Face,* 2·1 *mcd.* *Case* 3.—*Septic hand,* 10·4 *mcd.*

Tender Scar. Tenderness of scar in finger relieved.

S.D., Finger, 0·5 *mcd.*

Cut Nerves (2 *Cases*). *Case* 1.—Was radiated three weeks after suture of a completely divided musculo-spiral nerve to hasten the return of functional activity. Treatment apparently had the desired effect, and progress has continued to be satisfactory. Seven months after the accident he can extend his fingers and wrist, but the abduction of the thumb is weak, but improving.

S.D., front and back of arm, injured area, 16·4 *mcd.* 11 *hours.*

Case 2.—Radiation for extensive scars in the wrist, in which the ulnar nerve had been divided seven years before, was followed by some increased movement in the hand, due to loosening of the scar tissue.

S.D., Wrist and forearm, 20·0 *mcd.*

Sciatica. (2 *Cases.*)

Case 1.—Radiation relieved the pain almost at once.

S.D., gluteal region, 22·6 *mcd.* 24 *hours.* *A.A.* 2 × 16 *cm.*

Case 2.—Very crippled with sciatica. Was able to sleep all night when the radium was applied, which he stated he had not done for months. He left hospital for the country after five days' treatment very much improved, and has not been heard of since.

S.D., Hip, thigh, upper part of calf, 106·4 *mcd.* 105 *hours.*

Sepsis. (6 *Cases.*)

Acute (2 *Cases*). *Case* 1.—Was given a small dose of radiation on account of a "flare-up," accompanied by pain and swelling in the elbow, following old gunshot wounds. The pain, inflammation, and swelling subsided with the formation of a good deal of pus.

S.D., Elbow, 1·33 *mcd.* 32 *S.T.* *A.* 2 × 16. 44 *hours.*

Case 2.—Was admitted to hospital with a very swollen arm about three weeks after a septic cut in the finger. Instead of making numerous incisions, which would have been justified by the infiltrated condition of the tissues, I tried a small dose of radiation. This very soon relieved the pain, and reduced the swelling, and the patient made an exceptionally quick recovery, without surgical interference. Poultices and stupes were not employed, the limb simply being kept at an equitable temper by the use of cotton wool.

S.D., Arm and forearm, 3·8 *mcd.* 6 *days.* *A.A.* 2 × 20 *cm.* *Later,* 7·8 *mcd.* 17 *hours.*

Chronic (4 *Cases*). *Case* 1.—Was a septic finger of six weeks' standing, which cleared up quickly after radiation.

S.D., Finger, 0·38 *mcd.* *A.A.* 2 × 2 *cm.* *F.* 0·5 *Pb. d.* 3 *mm.* (0·9 *mcs. in old emanation capillaries strapped to finger for three days*).

Case 2.—Septic big toes; much improved when last seen. Pain gone.

S.D., round free part big toe, 4·1 *mcd. each.* 24 *and* 30 *hours.* *A.A.* 2 × 12 *cm.* *d.* 15 *mm.*

Case 3.—Was apparently not much helped by the dose given. Temporary increase of discharge.

S.D., along back and front finger, ·09 *mcd.* 45 *minutes.* *d.* 15 *mm.* *A.A.* 2 × 8 *cm.*

Case 4.—Had a septic condition of his hand of six years' standing, which would break down for a fortnight and heal again for another fortnight, and seriously interfered with his duties. The condition was said to arise from a ganglion which had been opened. The condition completely cleared up after radiation. The skin round the discharging area, which was much thickened, has become normal in appearance, and the patient has been completely free from any trouble, now for five months.

S.D., Palm Hand, 1·5 *mcd.* 2.*T.*7 *and* 2.*T.*10 *strapped to hand.* 5 *hours* 10 *minutes.*

Sinus.

A patient with a sinus following empyema was treated. No obvious improvement after one radiation.

Spring Catarrh.

One eye was almost well, and the worse eye (right) was much improved, after three applications of radium on the everted lids. This patient will probably require further treatment. In previous years, three patients have been cured of a similar condition.

26*th October*—*S.D., beta rays, R. eye,* 0·07 *mcd.* 5 *minutes.* *L. eye,* 0·06 *mcd.* 4 *minutes* (122 *mcs.*).

23*rd November*—*S.D., beta rays, R. eye,* 0·12 *mcd.* 7 *minutes.* *L. eye,* 0·09 *mcd.* 5 *minutes* (147 *mcs.*).

13*th January,* 1927—*S.D., through closed eyelids, R. eye,* 0·8 *mcd.* 2 *hours.* *L. eye,* 0·5 *mcd.* 1¼ *hours.* 6.*R.C.* *A.A.* 15 × 0·75 *cm.* *F.* 1·5 *Pb.*

Syphilis. (2 *Cases.*)

Case 1.—Pain and specific stricture of rectum somewhat relieved. Patient required colostomy subsequently.

S.D. and I.D., Rectum and anus, 10·0 *mcd.*

Case 2.—Several deep ulcers on this patient had been cured previously by radiation, but this ulcer was not so benefited, but subsequently healed after operation and radiant heat treatment.

Tonsilitis. (1 *Case.*)

Course of diseases apparently cut short.

1·0 *mcd.* 1 *hour. Area,* 2 × 7 *cm. against skin.*

Tuberculosis. (15 *Cases.*)

Abscess (1 *Case*).—Had been of long standing, but it finally healed after treatment this year.

S.D., 1 *mcd., in* 14 *days.*

Bladder (2 *Cases*). *Case* 1.—Radiation had apparently no decided effect in this case.

S.D., over bladder, 19·4 *mcd.* 2 *days.*

Case 2.—This patient had serious lung trouble, as well as extensive disease in both kidneys and bladder, accompanied by pain. For a couple of months before treatment she was getting severe rigors, with high temperature, every three or four days. After radiation, her temperature came down to normal, the rigors completely stopped, and the urinary condition ceased to cause her discomfort. She subsequently died from her lung condition.

S.D., Bladder, spleen, front and back of both kidneys, 55·7 *mcd.* 9 *days.*

Glands (1 *Case*).—Pain, and some of the swelling in this case of massive glands in both sides of the neck disappeared after radiation, and the patient's general health was much improved. Has been lost sight of.

30*th March to* 3*rd April—S.D.,* 52·2 *mcd. A.A.* 10 × 2 *cm. Two applicators with crossfire in three positions each.*

22*nd to* 27*th June—S.D.,* 48·0 *mcd. A.A.* 2 × 6 *cm.* 5.*P.*

Lungs (2 *Cases*). *Case* 1.—An army pensioner. Was reported last year, and had been given nine treatments altogether since 1920. On the last occasion two applicators, containing 1 mg. of radium each, were continuously applied from the 1st December, 1925, to 7th July, 1926—one in front and one at the back of the chest, at a distance of 5 mm. They were moved systematically every three days, the cycle taking about six weeks. He received approximately a dose of 10,000 mg.-hours in 207 days. This patient left hospital in very fair condition, and when seen two months later at a Pensions Board had become fat, and appeared in good health. No crepitation or cavity was found by the medical specialist. The first occasion on which he was treated he had a hectic temperature for over a year, and after a week's radiation it became normal for the first time, and remained so for several months. He had had several small, and some large, hæmorrhages, and the opinion expressed by the physician in charge of this case, and most emphatically by the patient himself, is that he owes his life to radiation.

S.D., front and back, both sides of chest, 73·02 *mcd.* 207 *days.*

Case 2.—Also had a tuberculous bladder, temporarily improved and crepitation diminished. Patient subsequently refused further radiation, and died with extension of the disease, though the apex radiated, I understand, remained free from crepitations.

S.D., Apex R. lung, 19·7 *mcd.* 17 *days.*

Lupus (5 *Cases*). *Case* 1.—With extensive disease of the face improved, but not cured.

Case 2.—With lupus of the ear, lost sight of.

Case 3.—With lupus on the surface of the nose, almost completely cured by one treatment. Second treatment recently.

S.D. (1), 0·4 *mcd.* 6.*R. cells against ulcer.* (2), 4·8 *mcd. d.* 18 *mm. A.A.* 2 × 2 *cm. F.*, 2·5 *mm. brass.*

Case 4.—Extensive tuberculous ulceration of the mucous membrane of the nose, and lupus of the nose and cheek. Lupus completely disappeared, leaving a somewhat glossy appearance of the skin, while the mucous membrane condition was very much improved. Recently given a treatment in the nostril.

I.D., 6·3 *mcd.* 1.*T.*5 × 2.*P. each nostril, A.R.* = 4. *S.D., Nose, cheek, forehead, and chin.* (1)$_{\gamma}$ 4·1 *mcd. d.* 2 *mm.* (2), 21·4 *mcd. d.* 20 *mm.*

Case 5.—Local improvement in case of 20 years' standing, which involved the hands, and had eaten away the nose.

S.D., Cheek, 2·9 *mcd.* 8 *days.*

Joints (1 *Case*).—A patient with very extensive disease of the knee-joint, where the question of amputation was considered, has now healed. She had a number of sinuses in the neighbourhood of the joint. The patient is now going home in plaster. Radiation appears to have helped her a great deal, and hastened her convalescence. After each treatment the discharge was much freer for a few days, and then gradually diminished, till it ceased altogether.

S.D., round knee, 34·3 *mcd.* 137 *hours. d.* 50 *mms.*

Peritonitis (1 *Case*).—Was first treated six years ago as a girl of 12, when her condition was extremely serious, and appeared hopeless. She has been under medical observation ever since, and grew into a well-nourished young woman. She remained in perfect health till March, 1926, when she contracted a severe attack of influenza, and ran a temperature for about two months, when typical tumours of the abdomen and some thickening round the umbilicus was discovered. She commenced to improve as soon as treatment started, and was radiated in April, June, and December. She gained 21 lbs. in weight in seven weeks after the last treatment, and 7 lbs. in the next month. She now appears to be in robust health again, though slight thickening still remains round the umbilicus. Radiation had no effect on menstruation, which has remained normal.

January, February, April, July, 1921—*S.D., front of abdomen. Total,* 39·8 *mcd. d.* 8 *mms.*

April, May, 1926—*S.D., abdomen and spleen,* 58·1 *mcd.* 10 *days. d.* 20 *mms.*

June, 1926—*S.D., abdomen and spleen,* 24·5 *mcd.* 12 *days. d.* 20 *mms.*

December, 1926—*S.D., abdomen and spleen,* 61·9 *mcd.* 6 *days. d.* 20 *mms.*

Tubes and Ovaries (1 *Case*).—This patient's tubes and ovaries had been removed for tuberculous disease in the Rotunda Hospital six weeks before treatment. She was emaciated, very weak, and had six sinuses discharging from the operating wound in the abdomen, and some discharge from the vagina. Radiation increased the discharge very considerably, but relieved the pain.

The patient, so far, may be said to have made an uninterrupted recovery, and now, three months after treatment, she looks the picture of health, and had become fat, and, except for small scab at the site of two of the sinuses, the abdominal scar had healed soundly. Undoubtedly, the convalescence of this patient was hastened markedly by radiation, and it is very questionable if she had the stamina to recover without its help.

22*nd to* 29*th December—S.D., lower abdomen,* 19·3 *mcd.* 42 *hours.*

8*th to* 12*th January,* 1927—*B.D.,* 4·15 *mcd.* 1.*T.*4 *in each of* 5 *sinuses. S.D., lower abdomen,* 28·0 *mcd.* 72 *hours.*

Stomach (1 *Case*).—This patient, an old phthisis case, was radiated for a condition diagnosed as tuberculous ulceration of the stomach, but, though she was able to take solid food immediately following radiation for the first time for some weeks, she succumbed a short time afterwards.

S.D., upper abdomen, 60·9 *mcd.* 3 *days.*

Ulcers (*Septic*). (6 *Cases.*)

Case 1.—Mooren's ulcer of the cornea. This case improved at first, was subsequently operated on, and the investigation of this method of treatment was not followed through.

S.D., R. eye, 2·8 *mcd. L. eye,* 2·1 *mcd.* 6 *days. d.* 15 *mm.*

Case 2.—A perforating ulcer of the foot, following gunshot wound of the thigh, appeared to be temporarily improved by radiation, but was not healed when last seen.

S.D., Heel, 3·5 *mcd.* 19 *days. A.A.* 2 × 16 *cm. d.* 10 *mm.*

Case 3.—A small septic ulcer in the mouth; and Case 4, a small ulcer, following gunshot wound, in the arm; and Case 5, an ulcer of the leg; and Case 6, a varicose ulcer, all healed soon after radiation, which appeared to hasten the process of repairs, and certainly diminished tenderness.

Case 3.—*Ulcer Mouth.—S.D.,* 0·0075 *mcd. Bare emanation capillaries, containing* 1·0 *mc.* 30 *minutes.*

Case 4.—*S.D., Arm,* 0·53 *mcd. Beta rays,* 0·02 *mcd.*

Case 5.—*S.D., Leg,* 3·8 *mcd. A.A.* 6 × 2 *cm. d.* 15 *mm.*

Case 6.—*S.D., Leg,* 4·1 *mcd. A.A.* 6 × 2 *cm. d.* 20 *mm.*

Warts. (2 *Cases.*)

Both of these cases were cured by one application of radium.

Case 1.—*S.D., Wart, finger.* (1) *Beta rays,* 0·03 *mcd.* (142 *mcs. for* 2 *minutes*). (2) 0·54 *mcd.* 2.*T.*10. 30 *minutes.*

Case 2.—*S.D., Wart, finger,* 0·45 *mcd.* (122·5 *mcs. for* 3½ *minutes. A.A.* 1 × 1·2 *cm. d.* 3 *mm.*).

Deductions from the Year's Radium Work.

In reviewing the work of the year I find that, on the whole, I have been using greater doses for the treatment of cancer than heretofore, but in some cases, I believe, not with corresponding advantage; I allude particularly to those in whom the disease is far advanced, and where the treatment can only hope to be palliative. I think it is not a great advantage to radiate intensely large skin areas in the track of the usual lines of dissemination of the disease, especially at the time when extensive tumour formation and glands require urgent treatment. This is particularly noticeable in cases of cancer of the mouth. When I have treated by surface application, with perhaps 50 surface tubes, the floor of the mouth, both sides of the neck as far as the clavicles, employing "cross-fire," a very thorough radiation is obtained of all the structures in the vicinity, and the shock involved appears to "take it out" of the patient very considerably. It is possible that just as satisfactory results would be obtained in advanced cases by confining myself to the radiation of obvious disease, and by leaving more for nature to accomplish in dealing with stray malignant cells, than by attempting too much. I have used a surface dose of 70 med., or more, when possibly 40 med., especially for old patients, would have as good a palliative result. I find, also, with buried needles, that an obvious cancerous mass will stand a great amount of radiation, with pronounced beneficial effects, but when the needles are withdrawn to adjacent areas, where there is no induration to be felt—with the idea of increasing the efficiency of the treatment—these areas do not appear to accommodate themselves so well to the same dose, and become swollen, which swelling takes time to disappear, and may leave some thickening behind. In fact, there is no doubt that a malignant mass stands radiation far better than normal tissue, and that the "multiple foci of illumination" (Joly) with primary beta rays, obtained in the technique I employ, destroys the malignant cells in the mass without reaching and devitalising the healthy cells outside, as the tumour acts as a filter to absorb the primary beta rays, and as a distance piece to cause the gamma ray energy to be more uniformly distributed, and, therefore, to be efficient, without undue injury resulting in the vicinity of the tumour.

My contention is illustrated by some of the cases of breast cancer reported above. The lump in the breast of Case 3, the dimensions of which were $7 \times 8 \times 2{\cdot}5$ cm., was radiated by buried needles, the buried dose being 21·1 med. This area received a very considerable buried beta ray treatment as well as surface radiation, and yet appeared to do extremely well. In Case 4, a buried dose of 89·6 med. was used, in about five times the area of Case 3, in front and behind the pectoral muscles and in the axilla, the radium being inserted at the operation after removal of the cancerous tumour, gamma rays alone being used. The energy was mostly expended on healthy tissue, scattered about among which there were almost certainly some malignant cells, ready to form centres of fresh growth. The wound practically healed after operation and radiation, but a couple of months later a certain amount of sepsis occurred, the effect of which was more pronounced in the area debilitated by intense radiation. Though there is no appearance of recurrence, the sepsis persists, and there is considerable œdema of the arm. In Case 6, on the other hand, in which there was more extensive disease—in the ordinary sense, an inoperable tumour—the amount of energy dissipated during treatment, 86·6 med., is practically the same

as in Case 5, though the buried dose is actually much greater, as the patient received beta radiation from both the tubes and needles employed. Considering the extent of this patient's disease, the result has so far been very gratifying. I believe one-third of the buried dose given in Case 5 would have been more satisfactory, and I believe that this method of treatment immediately after operation has a great deal to recommend it. It should stimulate the recognised efforts of nature (see Sampson Hanley) to eradicate the disease. This belief is confirmed by the result obtained in a somewhat similar case treated in January, 1927, in which I inserted four gamma ray tubes and the radium needles into the breast and axillary region, after I had removed as much as possible of a sarcomatous growth which was adherent to the axillary vessels. The total buried dose was 27·9 med. in two positions for 27 hours, and the surface dose 111·0 med. The wound healed quickly, and there was no further trouble while the patient was under observation for about three months.

The rationale of combining radium treatment with operation was illustrated in a case reported last year, and has been dealt with more fully in a paper in the *Lancet*. Here, the foul-smelling fungating mass in the breast, an encephaloid carcinoma, with the radon tubes still in it, was removed after a dose of 26·7 med. had been administered. Area, 6×5 cm. The skin was brought together over this area, and healed by first intention, without sepsis, or, as far as I know, recurrence in the area. This patient was not sent back from the country for further radiation, but lived 15 months after radium treatment. The same line of treatment was followed in the first case of sarcoma, reported above, in which a brain tumour was removed as soon as possible after intense radiation. Incidentally, the experience in this case, and the case of orbital tumour, appeared to show that brain tissue will stand a large dose of radiation without much apparent injury.

That dosage has an important bearing on results is seen in the tonsil cancers reported. In Case 1, the right tonsil, which appeared to be less affected, received half the dose administered to the left, which responded markedly to the treatment, and looked almost well seven months later, while the right tonsil was still obviously diseased. In Case 2, a small dose healed up a small ulcer, but it recurred in four months, and did not respond satisfactorily to further radiation.

In connection with the radiation of non-malignant cases, many of these would not have been treated if radium had not been available. Once it is established that radiation, in suitable doses, hastens the repair of wounds, has a salutary effect on inflammation, sepsis, and chronic infections, like tuberculosis, and influences redundant fibrous tissue, which interferes with function, the scope of its employment in general practice and by specialists is enormous. Nevertheless, radium treatment cannot be recommended as a routine treatment, except in special circumstances, as its general use involves too much responsibility, labour, and risk, not to mention expense. At the same time, its routine employment in cases such as tuberculous peritonitis, has always been justified, in my experience, by the immediate amelioration of symptoms, and the comparatively rapid convalescence which follows radium treatment.

Finally, it is not possible to draw very definite inferences from one or two cases of a particular condition treated for the first time, as Addison's Disease and gangrene were treated this year; but it is not unreasonable to expect that if radiation helps the patient to combat tuberculosis in one locality it may do so in another; and that if the circulation in a limb is improved after

radiating the nerves and blood vessels involved in a trauma, the result of a bullet wound, it may help to restore the blood supply, which is deficient from some other cause.

In conclusion, I wish to express my indebtedness to Dr. Poole and his Laboratory Staff at the Royal Dublin Society for their invariable courtesy, and the accuracy of their details, when supplying emanation. I wish to thank those who have kindly referred patients to me, particularly when they have also assisted me in carrying out radium treatment by their special knowledge of throat, brain, or gynæcological surgery; and my Residents at Dr. Steevens' Hospital, who have always helped me most conscientiously in my radium work.

PAPERS DEALING WITH RADIUM TREATMENT PUBLISHED SINCE THE REPORT OF THE YEAR 1926.

14. The Rationale of the Treatment of Middle Ear Deafness, with a Preliminary Report of its Effect in Chronic Hyperplastic Otitis Media. Walter C. Stevenson and T. G. Wilson. Irish Journ. of Med. Science, November, 1926. Med. Press and Circular, November 10th, 1926.
15. The Treatment of Middle Ear Deafness by Radium: Its Rationale and Technique. W.C.S. and T.G.W. The Journal of Laryngology and Otology, February, 1927.
16. The Rationale and Technique of Combining Operation with Radium Treatment in Cancer. W.C.S. The Lancet, 4th December, 1926.
17. Radium in Cancer. W.C.S. Irish Journ. of Med. Science, June, 1927.

REPORT OF RADIUM TREATMENT CARRIED OUT IN 1926.

BY MAURICE R. J. HAYES, F.R.C.S.I.

DURING the year 1926 forty-six cases were treated with radon. These comprised:—

Rodent Ulcers	25
Cutaneous Epitheliomas	5
Oral Carcinoma	5
Uterine Carcinoma	4
Recurrent Cancer of Breast	2
Paget's Disease of Nipple	1
Keloids	2
Chondroma of Sacrum	1
Hairy Pigmented Mole	1

All were treated with radon supplied by the Royal Dublin Society. My thanks are due to Dr. Poole and his staff in the Society's Laboratory for their unfailing courtesy and their readiness to facilitate me, sometimes on short notice, with the supply of emanation.

The technique of the treatment of rodent ulcers seems now to be established

so satisfactorily as to demand no further consideration, except in so far as it may serve as a guide to determining the particular *quality* and the *quantity* of radiation which will produce the most beneficial effects on other types of cells which have a greater degree of malignancy.

Of the many besetting difficulties in the management and treatment of malignant disease, an outstanding problem is whether the patient should be told that he has carcinoma. Tradition and policy answer "No." To tell most people that they are suffering from cancer is equivalent to a sentence of death. On the other hand, most patients would be more zealous in co-operating with the doctor, and in returning at the specified intervals for treatment and inspection if they realised that they were suffering from the malady, and that there was a reasonable prospect of curing them if they attended regularly.

I consider that each patient should be informed of his condition, for I am convinced that numerous patients are lost because the serious nature of their malady is concealed from them, and, consequently, they are not alive to the necessity for periodic inspection, and they are haphazard in their attendances. One's experience is that, however sensitive patients may be temperamentally, when they realise the inevitable, they are not unduly disturbed, except by the regret that if they had "only known," they would have been more particular and more regular in coming back for treatment.

Rodent Ulcers.

In this series of twenty-five cases treated during 1926, 21 were primary, and 4 were recurrent. Of the twenty-one primary cases, one ulcer had been present for three months, and one for twelve years, before advice was sought. Most elderly people do not trouble about treatment until ulceration takes place, unless the ulcer appears near the eye, when, through fear of loss of vision, early advice is sought; or, if on the nose, for æsthetic reasons.

Unless the ulcer has extended to the underlying bone or periosteum, ulceration does not materially affect the prognosis, or the cosmetic result, except when there is already gross loss of tissue, as may occur at the ala nasi, or at the eyelids.

Of the four recurrent cases, one reappeared after six years, one after five years, one after four years, and one after seven months. All of these were marginal recurrences. The early recurrence in the case which reappeared after seven months was undoubtedly due to under-dosage, as it was not possible to give the requisite dose in the first instance.

Technique.

To superficial ulcers, a surface application of 60 millicurie-hours per square centimetre is given, through an aluminium filter 2 mm. thick, dry scales, or incrustations, having been previously removed.

When the margins of the ulcers are very much raised and infiltrated radon needles are embedded under the local anæsthesia, and a dose of 60 mc.-hours *per* cubic centimetre is applied. This application is followed by a mild reaction, which appears in from ten to fourteen days; complete healing takes place in from four to six weeks later. When the ulcer has broken down, the application of an antiseptic, such as 1 per cent. solution of mercurochrome, or a simple dressing with boric or zinc ointment, is the only after-treatment which is necessary.

With accurate dosage and proper technique, there is no doubt but the

radium treatment of rodent ulcers is superior to all others. The cosmetic result cannot be surpassed: the scar is soft and pliable, and it is scarcely perceptible; there is no unsightly contraction of scar tissue, and no tendency to keloid formation, and, if the dose is not over-estimated, telangiectases do not form.

Cutaneous Epitholiomas.

1. Male, aged 68. A red, cauliflower-like tumour appeared as a wart in the external auditory meatus, and after three months it was as large as a hazel nut, and filled the hollow of the external ear. Needles were embedded in the tumour, dose 240 mc.-hours, followed four days later by a surface application of 306 mc.-hours filtered through 2 mm. of brass. When seen one month later the growth had completely disappeared, and there was no recurrence a year afterwards.

2. Male, aged 81. Six months after excision an epithelioma recurred in the external ear. The growth was soft and ulcerated, with a copious moist discharge, and about the size of a hazel nut.

Following a surface application, 70 mc.-hours 2 mm. aluminium filter, to the front of the ear, and 65 mc.-hours to the back, the ulcer disappeared.

The patient returned to the country, and has not been heard of since.

3. Male, aged 30. A malignant ulcer appeared at the angle of the mouth in tubercular scar tissue, and there was a nodule about the size of an orange-seed midway between the skin and mucous membrane of the cheek.

A surface application, 311·5 mc.-hours 2 mm. brass filter, was given. The next day the ulcer and the nodule were curetted, and while the wound was open three needles were embedded, a dose of 157 mc.-hours being given. When seen nine months later, the whole area was covered by a healthy pliable scar, and there was no evidence of recurrence.

4. Male, aged 65. An epitheliomatous ulcer about the size of a shilling appeared on the cheek, and it was growing rapidly on the site of a soft wart, which had been excised three months previously. The ulcer was very moist, and discharging freely. Six needles were embedded around the margins of the ulcer, and simultaneously a surface application through a 2 mm. aluminium filter was given, the total dose being 541 mc.-hours. Two months later the ulcer was healed.

5. Male, aged 70. Following the razor wound of a birth mark (angioma), an epitheliomatous ulcer was growing rapidly on the cheek. It was about the size of a two-shilling piece. The glands at the angle of the jaw were enlarged. Needles were embedded in the ulcer; dose, 300 mc.-hours.

Next day a surface application was given; 2 mm. brass filter, 300 mc.-hours. The glands at the angle of the jaw received a surface dose of 936 mc.-hours, 2 mm. brass filter.

The patient was recently treated, but his doctor reported that he was very much improved two months later.

Oral Carcinoma.

1. Male, aged 54. Rapidly growing epithelioma of posterior half of floor of mouth, with enlarged submaxillary glands. Four radon needles embedded, dose 420 mc.-hours, followed by a surface application intra-orally, 260 mc.-hours in brass tube 1 mm. thick. The submaxillary glands were cross-fired, three areas: 2 mm. brass filter 1·5 cm. distance. The total irradiation given was 3,627 mc.-hours. When seen a month later the primary ulcer was smaller, but

it had extended backwards towards the base of the tongue. The submaxillary glands were still enlarged, and there was œdema of the soft tissues of the neck.

High voltage X-ray treatment was then given ·09 Angström units 5 zn. and 2 mm. aluminium. F.S.D. 30, Field 12 by 10. 110 per cent. U.S.D. Palliation and relief of pain followed, but the patient died from the disease three months later.

2. Male, aged 52. Recurrent carcinoma of base of tongue and floor of mouth 16 months after local excision: enlarged submaxillary glands.

High voltage X-ray treatment was first applied. The following day radon needles were embedded in the ulcer; dose, 345 mc.-hours. Amelioration followed, but three months after treatment patient was not doing well. No recent report.

3. Fungating carcinoma of middle third of lower lip, 1 inch long by ½ inch wide.

Radon needles embedded 165 mc.-hours, followed by a surface application 145 mc.-hours 2 mm. aluminium filter. The right and left submaxillary glands, which were but very slightly enlarged, were given 1,600 mc.-hours: 2 mm. brass 1·5 cm. distance.

Seven months later the ulcer was healed, except for a small fissure in the centre, which oozed a little serum. The submaxillary glands were not enlarged. A surface application 60 mc.-hours with 2 mm. aluminium was given to the lip. This patient is doing very well. There is no ulceration, and no evidence of disease now.

Sarcoma of Tonsil.

Female, aged 47. The tumour was about the size of a walnut. It extended inward beyond the fauces and forwards to the hard palate. Radon needles were embedded in the tumour; dose, 770 mc.-hours.

This was followed by high voltage X-ray treatment ten days later—80 per cent. U.S.D. One month later the laryngologist informed me that the tumour had completely disappeared.

I saw the patient again eleven months after treatment, when the fauces were quite healthy, and there was no trace of recurrence. She then had symptoms of chronic gastric ulceration, and at operation the macroscopic appearances of the stomach suggested malignant disease of that organ, but this was not confirmed microscopically.

Uterine Carcinoma.

Four cases were treated. All were very advanced, and inoperable. Radium was employed to palliate the disease.

1. Age 50. Squamous epithelioma of cervix. Very advanced. Hæmorrhage and fœtid discharge for one year. Vaginal vault completely involved. Bladder and rectum adherent to growth. Cachectic. Following treatment, discharge was diminished, but general condition was so bad that removal to Hospice for Dying was advised.

2. Age 51. Sanious discharge for six months. Latterly profuse, fœtid, and frequent hæmorrhage. Crater cavity replacing cervix: anterior and posterior vaginal walls indurated and sloughing.

Following treatment, discharge and bleeding diminished. Died six months later from metastases.

3. Age 42. Nullipara. Profuse hæmorrhage and discharge, 12 months.

Cachectic. Advanced adenocarcinoma of cervix. Bladder and rectum adherent to growth.

Ten days after treatment patient left hospital much improved. No hæmorrhage, very slight discharge. Told to report within two months—did not return.

4. Age 42. Multipara. Menstruation normal till six months before seeking advice. Complaining of constant discharge, and hæmorrhage. Cervix destroyed, and bleeds readily. Adenocarcinoma of cervix, widely infiltrating vagina and parametrium. Left hospital two weeks after treatment. Discharge considerably diminished. No pain. Told to report within two months, but did not do so.

Mode of Application.—Cases 1, 2, and 3. Radon was packed in brass tube, wall 1 mm. thick, covered by rubber tubing. Dose, 1,150 to 1,200 mc.-hours. In case number 1, the tube was laid on the ulcer, as the cervical canal could not be identified. In Cases 2 and 3, the tube was placed in the cervical canal, and the vagina was closely packed with gauze. In the fourth case, needles were embedded about 1 cm. apart around the cervix. In all, the vagina was closely packed with gauze. No undesirable complications followed the treatment in any of these cases. There was no irritation of the bladder or rectum, and the systemic disturbance was negligible.

In all these cases the prognosis was very bad. There was no prospect of cure in any of them, and radon was applied only as a palliative in order to relieve pain and other distressing symptoms, which it undoubtedly accomplished successfully.

The value of radium or its emanation as a palliative in advanced carcinoma of the cervix uteri is unquestionable. In the writer's experience during the past eleven years he has never known it to fail in relieving the most distressing symptoms, and for this reason its employment is always justified, no matter how advanced the disease may be. It will give more lasting relief and more comfort to the patient than any other known remedy. When properly applied, it is absolutely safe: it is certain and rapid in its action—relief will be obtained within a week—and the pain incidental to its application is trifling. If radium did nothing more than this, its place in gynæcological treatment is amply justified.

Mammary Carcinoma.

1. Male, aged 86. Paget's disease of nipple with secondary nodules in adjacent skin.

Using a filter of 2 mm. of brass with a layer of cardboard 2 mm. thick the applicator was laid on the skin. It was left on until 2 millicuries *per* square centimetre of skin area had been dissipated. The whole of the affected region was irradiated in this manner. Treated in January, 1926, and apparently cured.

2. Female, aged 43. Two years after removal of breast there was an excavated ulcer 3 cm. in diameter to the left of the sternum, in the third interspace. Another smaller ulcer was situate over the anterior end of the fifth rib. The surrounding skin was tense, and adherent to the ribs, and the pectoral muscles had been removed. The subcutaneous tissue was very scanty. A radiogram of the chest showed secondary deposits in both clavicles.

Needles were embedded in the margins of the ulcers, the estimated dose being 60 mc.-hours per square cm. of tissue. This was followed by applications, 1·5 cm. distance, filtered through 2 mm. of brass to each ulcer, to the clavicles,

and to the supraclavicular regions front and back, nine millicuries being dissipated during the application to each separate area; the dimensions of the applicator and the size of the "fields" were 5 cm. by 3·5 cm.

Two months later the smaller ulcer had healed, and the larger ulcer was very much reduced in size and depth. The supraclavicular glands were much smaller. A new crop of nodules had appeared to the left of the umbilicus, but the patient's general health was good. Radon was again applied as before, but no needles were embedded. When seen five months later the larger ulcer had almost healed, and the nodules to the left of the umbilicus had disappeared. The supraclavicular glands were fibrotic and "shotty." Patient was slightly anæmic, but there was no clinical evidence of thoracic or abdominal metastases. Patient told to return for treatment, but did not do so. Not heard of since.

4. Female, aged 55. Three years after amputation many scattered nodules appeared in the left pectoral region, which was uniformly irradiated in five separate areas, 2 mm. brass filter, 1·5 cm. distance, 9 mcs. being dissipated on each area. When seen two months later the nodules had disappeared after dosage applied as above. Five months after first treatment new cutaneous nodules appeared below the clavicle, in the mid-axillary line over the lower ribs, and in a short time the lateral aspect of the thorax was extensively involved. These recurrences were kept in check by further applications, but it was manifest that the disease had extended to the liver, and the patient died eleven months after radium treatment commenced. Of the fragile, asthenic type, with little power of recuperation, nothing more than palliation, and the prevention of ulceration, was expected in this case, and this hope was realised. There was no cutaneous ulceration, and she was spared much pain, discomfort, and suffering.

Chondroma of Sacrum.

Following partial excision of a very large chondroma, which arose from the anterior surface of the three lower sacral segments, a brass tube 1 mm. thick, containing 66 mcs. radon, was packed in the sinus. Dose, 1,188 mc.-hours. This was supplemented by deep X-ray treatment, 110 per cent. U.S.D. delivered to the neoplasm. Eight months after treatment there was no apparent increase in the size of the growth.

Painful Keloids.

1. Three months after excision from the outer border of the nail of the third right finger the keloid recurred. There was considerable induration, and the matrix was thickened. A surface dose of 70 mc.-hours, 2 mm. al. filter, was given. Three months later the keloid and induration had disappeared.

2. Two and a half months after excision of a painful corn from the outer border of the fifth toe, the scar was indurated, and very painful. Only a slipper could be worn, and, before having the toe amputated, it was decided to try the effect of radium. A surface application, 50 mc.-hours, 2 mm. al. filter, was given. The induration and pain disappeared, and six weeks later the patient was quite well.

Pigmented Hairy Mole.

Boy, age 13 years. Pigmented mole, $1\frac{1}{2}'' \times \frac{3}{4}''$, above right eyebrow since birth. The skin was bronzed, and covered by a luxuriant growth of stout black hair, like horse hair, which was very disfiguring. A surface application of 100 mc.-hours, 2 mm. al. filter, the surrounding healthy skin being protected

by lead-rubber, to one half of the mole, was followed the next day by an application of 88 mc.-hours, with the same filtration and protective rubber, to the other half. The area of the patch which was treated being eight square centimetres, the total dose administered was 25 mc.-hours per square centimetre, added to which was the over-lapping effect of the two adjacent areas exposed.

When seen six months later the hair follicles were atrophied, and there was no pigmentation, except along the upper margin, where some strong hairs and pigment were still present. After repeating the treatment as above, these also disappeared. This patient was treated in April, 1926, and his father informs me that the cosmetic result is entirely satisfactory.

No. 40.

INHIBITION OF POLLEN GROWTH BY LIVING TISSUE EXTRACTS.

By P. O'CONNOR, B.Sc., A.R.C.Sc.I., Botanical Laboratory, University College, Dublin.

[Read May 24. Published separately July 22, 1927.]

In the Spring of 1924, an effort was made to determine whether malic acid has an influence on the direction of growth of pollen tubes of angiosperms. Two sets of glass capillary tubes were filled with media, consisting respectively of one per cent. and one-half per cent. of malic acid in a mixture containing six per cent. each of gelatine and cane sugar. The filled tubes were washed in water, and dropped into dishes containing shallow layers of a similar mixture of gelatine and cane sugar (not containing malic acid), with which had been mixed the pollen of *Scilla verna*, at a temperature of 20–25° C. The directions taken by the pollen tubes in the immediate neighbourhood of the malic acid were not seen to have been influenced by the presence of the malic acid. Several trials were made, always with the same result.

In 1925, and again in 1926, these experiments were repeated with the pollen of Scilla, Lilium, and Tulipa; and, the results being the same as in 1924, it was concluded that malic acid does not influence the direction of growth of pollen tubes of angiosperms.

At the end of March, 1927, an attempt was made to discover whether there is any chemical stimulus, other than that of the presence of cane sugar, affecting the direction and the character of the growth of pollen tubes. On March 31st, a medium was made up, consisting of six grams of gelatine and six grams of cane sugar per hundred grams of water. Thin layers of this were poured into two Petri dishes, into each of which was also stirred some pollen of Daffodil (*Narcissus pseudo-narcissus*), when the temperature had fallen to about 25° C. After one hour portions of the jelly were removed with a scalpel, and into the furrows thus formed was poured, by means of a pipette, a medium made up by adding a mixture of twelve per cent. each of gelatine and cane sugar, at a temperature of 30° C., to an equal volume of a watery extract of stigmas of Daffodil (made over-night), at the same temperature. On April 1st the pollen tubes in the vicinity of the stigma-extract did not appear to have their direction of growth affected by it; for, while some entered the stigma-extract medium, others continued to grow beside it and away from it, indifferently. The tubes continued to grow on April 2nd, and remained unaffected in direction.

It was decided to compare the effect on pollen tube growth of the proximity of watery extracts of stigmas of the same and of other species. Accordingly, on April 1st, thin layers of a medium containing six per cent. each of gelatine and cane sugar were poured into Petri dishes, and pollen of Tulipa was shaken into each, and distributed in the medium at a temperature of about 25° C. After one hour, additions were made as before of media made up by mixing equal parts of twelve per cent. gelatine and cane sugar mixture and watery extracts of the stigmas of Crocus, Daffodil, Wallflower (*Cheiranthus cheiri*), and Tulipa.

After seventeen hours, the pollen tubes near the Tulipa stigma-extract did not show a growth superior to that of those more remote; the pollen grains in the vicinity of the extracts of Narcissus and of Cheiranthus stigmas were either without tubes or the tubes, which had grown haphazard towards these extracts, had all stopped short at a uniform distance from them of about 0·1 mm.; the Crocus stigma-extract, which was very dilute owing to the infusion having been made with only two stigmas, did not seem to have any marked effect on the pollen tubes which grew beside it. At noon, on April 4th, about 90 per cent. of the pollen in the gelatine and sugar medium had germinated, and the tubes had reached an average length of 1·5 mm.; the pollen in the immediate neighbourhood of the extracts of the stigmas of Cheiranthus and of Narcissus had either failed to grow or had produced tubes which still remained at the same distance from the extracts as on April 2nd. The tubes in the immediate vicinity of the Crocus extract were now observed for the first time to have been affected by the proximity of the extract, their average length (about 0·3 mm.) being markedly less than that of those more remote.

On April 2nd, it was decided to compare the growths of the pollen of Tulipa and of Narcissus in the media remaining from the trials started on April 1st, consisting of the extracts of the stigmas of Tulipa, Narcissus, Cheiranthus, and Crocus, with additions of equal volumes of twelve per cent. gelatine and cane sugar mixture. After 24 hours, about 90 per cent. of the Tulipa pollen grains had produced tubes of an average length of 1·5 mm. in the Tulipa stigma-extract; not more than 50 per cent. of the Tulipa pollen grains in the Crocus stigma-extract had produced tubes of a maximum length of 0·3 mm.; the maximum length of the tubes, which grew from only about 30 per cent. of the Tulipa pollen grains in the Narcissus stigma-extract, was 0·15 mm.; in the Cheiranthus stigma-extract there was not any growth. At the end of 48 hours, the Tulipa pollen tubes had reached a length of 1·8 mm. in the Tulipa stigma-extract, while in the other media their condition remained substantially the same as on the preceding day. The Narcissus pollen responded in a somewhat similar manner to that of Tulipa, except that, while it produced tubes of about 1·5 mm. in length in the Narcissus stigma-extract, it made practically no growth in the Tulipa stigma-extract. It is clear, therefore, that the stigmas of the flowers of each species contain soluble substances, which are poisonous to foreign pollen.

In view of these toxic effects of the extracts of the stigmas of foreign species on the growth of pollen, the attempt to elucidate the conditions which determine the direction of growth of pollen tubes was temporarily abandoned, and further investigation of the inhibition and its causes was undertaken. Owing to the production of rather sinuous, meandering tubes by the pollen of Tulipa, and in a more marked degree by that of Narcissus, the concentration of the sugar solution for further investigation was reduced to three per cent., and the same reduction in the strength of the jelly was found suitable. At this concentration the Narcissus pollen grew well, and produced comparatively straight tubes; but it was found that the Tulipa pollen sometimes produced only indifferent growths from a small percentage of grains, as heretofore. Hitherto, the gelatine used had been selected indifferently from either of two samples in stock; and it was noticed that the pollen (more especially that of Tulipa) grew the better when the gelatine for its culture was selected from one of these samples, and that abnormal tubes were produced when gelatine from the other sample was used. Consequently, only the gelatine from the stock in which the superior growth was obtained was used, until that supply became exhausted.

It was found impossible, in concentrations of cane sugar and gelatine varying from six per cent. to one-half per cent., to induce the growth of the pollen of Cheiranthus and of Taraxacum.

Commencing on April 4th, extracts were made for 24 hours of stigmas of Salix and of Narcissus; and, on April 5th, these extracts were made up to three per cent. with gelatine and with cane sugar, and half of each mixture was boiled. The boiled and the unboiled media and also a three per cent. gelatine and sugar mixture in water were poured into dishes, into sets of which were stirred separately the pollen of Narcissus, of Salix, and of Tulipa. It was found that the boiled extracts inhibited the growth of the pollen tubes of Tulipa in a somewhat more marked degree than the unboiled; the same was true of the effect of Salix stigma-extract on the Narcissus pollen, and *vice versa*. The Narcissus stigma-extract did not seem to stimulate the Narcissus pollen, nor the Salix extract the Salix pollen, to growth superior to that which was produced in the medium made up with ordinary water. This experiment was repeated, except that the extracts were each boiled separately before mixing with the growth media. The results were the same. A similar series of experiments with Primula stigma-extract and the pollens of Salix and of Tulipa gave like results. The effectiveness of the substance, which in the stigma of each flower is toxic to foreign pollen, is not impaired by heating to 100° C.; that substance is not of the nature of an enzyme.

It was decided to inquire whether this inhibiting substance is universally present in all the cells of the plant. Accordingly, cultures were started of the pollen of Salix, Tulipa, and Narcissus in three per cent. gelatine and sugar, and in boiled and unboiled extracts of Narcissus stigma, Narcissus stem, and Tulipa stem, containing three per cent. gelatine and cane sugar. The stem-extract was found to be a more potent inhibitor of the growth of foreign pollen than that of the stigma. No doubt, this result was due to the greater concentration of the inhibitory substance in the stem-extract owing to the greater facility in providing adequate material. Again, the growths of foreign pollen in stem-extract were found to be more stunted in the boiled than in the unboiled media. In a subsequent trial with boiled extracts, some of which were thoroughly aerated after boiling, it was shown that the disparity in the growths in the boiled and in the unboiled extracts was solely a function of the relative amounts of oxygen in solution—the lower oxygen-content of the boiled medium tending to help in the depression of pollen tube production. Similar experiments were carried out with the pollens of Salix and of Narcissus, and boiled and unboiled extracts of Cheiranthus stem, Brassica leaf, leaves of Grasses, Pear leaf (*Pyrus communis*), Apple fruit (*Pyrus malus*), Tomato fruit (Lycopersicum), Elodea shoot, rind of Orange (*Citrus aurantium*), and pith and rind (separately) of Potato tuber (*Solanum tuberosum*), and with the diluted, and undiluted, boiled, and unboiled, juices of Orange and of Grape (*Vitis vinifera*), made up without the addition of sugar to the culture media. Pollen tubes were freely produced in the control cultures; and, while all swelled, not a single pollen grain produced a tube of greater length than its own diameter in the various extracts and juices, with the exception of the extract of Apple, which did not include that of the epidermis. In this latter case, about 50 per cent. of the pollen grains of Salix gave tubes of a maximum length of 0·15 mm. in 36 hours, during which 90 per cent. of the pollen grains in the control medium had made growths 0·9 mm. in length.

It may be well to indicate that, in every instance in which comparisons were sought of the growth of pollen in different media, all due and reasonable

precautions were taken to ensure, as far as possible, that each culture dish had a fair sample of the pollen employed. Thus, when Silax pollen was used, apparently suitable catkins were selected in number equal to the number of culture media, and a sample from each catkin was shaken into each dish, a beginning being made with every dish of the set in turn, and the others being treated in rotation. Similarly, six flowers of Narcissus or of Tulipa were selected for each set of six or fewer cultures, and a sample of the pollen from one stamen of each flower was introduced into every medium; and, in the cases when the number of cultures in a set exceeded six, at least two controls were set up.

A dense mixture was made of the pollen of Salix, Populus, Narcissus, and Tulipa in a three per cent. gelatine and sugar medium, and the growths were compared with the growths, during the same time, of pollen from the same sources in separate media of the same concentration. In both instances the Populus pollen failed to germinate. In no case were the tubes produced in the mixture by the pollen of Salix, Tulipa, or Narcissus of greater length than half that of those in the pure culture; and, the pollen of each species having remained somewhat segregated in clusters, the tubes produced were in many instances quite diminutive, and, in most cases, stopped short of entering the regions of the pollen clusters of other species. In this respect, however, the pollen tubes of Salix seemed to insinuate themselves best among the Populus pollen grains; and, on the whole, the Salix growth seemed to predominate. Also, the Salix tubes were mainly normal, while those of Narcissus and of Tulipa were of a monstrous type, frequently observed in milder inhibiting media throughout these experiments. But from these observations it is not sought here to draw any general conclusions, since the mixtures were promiscuous and empirical, and numerical or other quantitative comparisons were neither devised nor subsequently attempted.

From these experiments it is concluded that, in angiosperms, each species contains within each cell substances which are toxic to foreign pollen.

During the course of these experiments the possibility suggested itself that some of the iron of the cutting tool, which was used in the preparation of the early extracts, went into solution, and affected adversely the growth of the pollen in the medium subsequently prepared. This apprehension was allayed when extracts, made by crushing in porcelain, glass, and agate mortars without any contact with iron, proved to be not less toxic to pollen than those obtained by cutting the material into thin slices with a steel tool.

The possibility was considered of the acidity, or otherwise, of the extracts affecting the pollen tube development. Using Brom Thymol Blue and Brom Cresol Purple as indicators, the hydrogen ion concentrations of a variety of extracts were found to be as indicated by the following values respectively for pH:—Salix stigma, 7; Tulipa stigma,[1] 6·8–7; Cheiranthus stigma, 6·6–6·8; Cheiranthus stem, 6·6–6·8; Narcissus stem, 6·2; Tulipa stem,[1] 5·2–5·5. However, it was found that the pollen of Salix and of Narcissus grew equally well in three per cent. gelatine and sugar media of $pH = 5·0$ and of $pH = 6·8$. Within natural limits, it would appear that the hydrogen ion concentration of the medium is not an important factor in determining the growth or otherwise of pollen.

[1] These two estimations were confirmed, but were not repeated with new material. No doubt, the disparity would be accounted for by the fact that only a few stigmas of Tulipa were available for making the extract.

It was then sought to discover whether the inhibiting substances are diffusible through a permeable membrane. Extracts of stems of Narcissus and of Tulipa were prepared. Half of each extract was thoroughly boiled and filtered, and the remainder was put into a diffusion shell of parchment paper, which was immersed in an equal volume of water for 20 hours. Portions of the clear, diffused extracts were then diluted with equal volumes of water and with double their volumes of water, and made up as culture media of three per cent. gelatine and cane sugar concentration. These diffused extracts proved to be toxic to the pollen of Salix equally with the boiled and filtered extracts; and the pollen of Tulipa failed to produce tubes in the diffused Narcissus extract, and the pollen of Narcissus in the diffused Tulipa extract. These trials were repeated with the leaves of Brassica, Pyrus, and Grasses, the diffused extracts of which proved to be inimical to the growth of Salix pollen. The substances which, in the cells of angiosperms, are toxic to foreign pollen, are of a simple, diffusible nature, and their toxic properties are not destroyed by heating in solution to 100° C.

Using Carrot (Daucus), Beet (Beta), Artichoke (*Helianthus tuberosus*), Tomato (Lycopersicum), Orange (Citrus), and Grape (Vitis), and the pollen of Tulipa and of Fritillaria, it was found that heating for three hours at 100° C. before extracting the juice does not impair the growth-inhibitory properties of the sap of plants.

During the latter part of this work the idea repeatedly occurred that the osmotic pressure of the inhibiting media was a major factor in repressing the growth of the pollen. Such fears, however, were always dispelled by the considerations that the pollen of Tulipa grew moderately well in twelve per cent. cane sugar; that the extracts had been made with volumes of water usually in excess of the volumes of tissue employed; that these extracts retained their inhibitory properties in a modified, but distinct, degree when diluted often with five times their volume of water; that in all cases the pollen grains swelled in them, and showed no signs of plasmolysis; that incipient pollen tubes were frequently observed in them; and that, in every instance in which pollen was grown in an extract of the same species, the resulting growth was not materially different from that in a simple medium containing gelatine and sugar in the same concentration.

In all the extracts referred to in this account with the exception of those of stigmas, which were not tested for them, amino compounds were found to be present.

It was decided to examine animal tissues and products for the presence of these growth inhibitors of pollen. About half of the albumen of a fresh egg of the domestic fowl was diffused in a diffusion shell of parchment paper into an equal volume of water for 20 hours; the remainder, mixed with half its volume of commercial *liquor pancreaticus,* was similarly treated. A quantity of the same sample of *liquor pancreaticus* was put into a third diffusion shell, surrounded with three times its volume of water. The diffused extracts thus obtained were found completely to repress the growth of the pollen of two species of Fritillaria. Their effects on Tulipa pollen could not be gauged owing to the failure of that pollen to grow in the control medium also. The egg-extracts were found, when diluted nine times (the volume of the growth medium being ten times that of the volume of the egg albumen), to inhibit in a modified degree the growth of the pollen of Fritillaria; the tubes produced were comparatively short and abnormal in character. In this respect, there was no marked difference between the two egg extracts. Diffused egg albumen was found to retain its inhibitory properties unimpaired after boiling for one hour.

The information here obtained was forthwith turned to account. The gelatine, which had hitherto given unsatisfactory growths of Tulipa and other pollen, proved amenable to washing for 24 hours. In a medium made up with washed gelatine, pollen tubes were seen for the first time on 100 per cent. of Tulipa pollen grains, and the tubes continued to grow normally for two days. In all subsequent trials washed gelatine was used. Also, this consideration must be taken into account in evaluating the results hitherto obtained.

Fresh blood-serum from a bullock was next subjected to the usual series of tests, except that in this case the growth media contained only two per cent. each of gelatine and of cane sugar. The pollen of Tulipa and of Fritillaria failed completely to produce tubes in the serum medium and in media containing diffused serum-extract and extract which had been boiled for one hour. These results were confirmed by repetition.

The blood of a sheep was similarly examined, with like results.

Fresh cow's milk was diffused into water for 20 hours. The extract was boiled and filtered, and made up as a two per cent. gelatine and cane sugar medium in dilutions corresponding, respectively, to two and five times the original volume of milk. In neither case was the growth of Tulipa pollen completely repressed. After eight hours the tubes which grew in the more dilute extract were equal in length to those in the control culture, but they were produced from only about 50 per cent. of the grains as against about 90 per cent. in the control. In the less dilute extract, there was a similar depression in the number of the tubes formed, and these remained quite diminutive. These results were confirmed by repetition.

Amino compounds were found in the various inhibitory extracts of egg, of blood, and of milk.

Complete inhibition of the growth of the pollen of *Fritillaria lutea* was observed to be produced in watery extracts of tissues of *Fritillaria pyrenaica, F. lanceolata* (var. *gracilis*), and *F. meleagris,* and the growth of that pollen was depressed to an almost negligible amount by an extract of *F. Elwesii* (*F. acmopetala*). This is evidence that the sap of one species is toxic to the pollen of other species of the same genus.

It is concluded that the fluids of each species of plant and of animal contain a simple, diffusible substance of a specific character which is toxic to the protoplasm of all other species, that this substance is not destroyed by boiling, and that it is probably an amino compound.

It may be well to point out that the reasons for suggesting that the inhibiting substances are amino compounds do not rest solely on the rather slender experimental evidence obtained during the progress of these tests. It is rather on general biological considerations that this suggestion is made. Until the true nature of these compounds is clearly established, it may be convenient to refer to them as a class by a definite name. The word "Toxin" suggests itself; but that sounds rather formidable, in view of the generally virulent nature of the substances which it designates. Accordingly, as a temporary expedient, it is suggested that the name *Speciamine* be applied to this class of compound until its exact nature is established.

Though the reciprocity is notoriously far from mutual, Fungi and Bacteria enjoy in varying degrees immunity from the otherwise general toxic effects of the sap of independent organisms. Furthermore, such foreign sap of a specific kind seems essential to the existence of many of them—the obligate parasites, whose protoplasm is attuned to the speciamines of the object of their choice. In the vast number of instances in which a particular parasite is found associated

only with a definite host, it must be concluded that the speciamines of all other possible hosts are antagonistic to the anxious intruder. Doubtless, in plants at least, the speciamines constitute, apart from the mechanical protective tissues, the chief natural defence against invasion.

It is not proposed here to discuss at length the many considerations of academic and practical interest which suggest themselves on examination of the foregoing principles. Perhaps chief among these is the possibility that the sap may be the source of mutation, and the protoplasm largely its instrument in this respect. The conclusion is imperative that the sap or other fluid of each species is the custodian of the identity of the race during the reproductive stages. The sap of each species is just as specific as is the protoplasm; and the differences in chemical composition between the saps of different species must be at least as clear—if comparison of such diverse qualities may be permitted—as the morphological differences which constitute the basis for their separation by the biologist. Of the two, it is believed that the chemical difference is the more fundamental, and that anatomical differences are mere expressions of this. In a cell, sap and protoplasm must be regarded as counterparts, each of the other. It is not justifiable, then, to assign to the protoplasm, or to any part of it, a dominating *rôle*; for, just as reasonably might the position of prime importance be allotted to the sap. No doubt, it would be more correct to regard them as a compatible, interdependent pair of units, for the harmonious working of which a delicately adjusted chemical relation must be maintained. In such circumstances, a considerable change in the composition of the sap, such as would be effected by the introduction of an appreciable quantity of the fluid of another species, would completely derange the balance; but, while not sufficient entirely to upset the harmony, minute quantities of foreign sap might conceivably be introduced into the system without more serious results than the production of abnormal growths, or other types of change. Such monstrous growths of pollen tubes have been observed repeatedly in the course of these experiments under the influence of dilute foreign sap. The suppression of growth in length was accompanied frequently by an increase in diameter to double, or even three times, the normal as spherical or clavate swellings. The volumes of units of the latter type were estimated sometimes to equal those of the tubes of normal elongation produced in control media in the same time. Mutations in Fungi of established pedigree have been observed, among others, by Schiemann (1), and F. Chodat (2). The latter, following De Vries (3), regards mutation as an expression of a pre-existing tendency in this direction, which is only evoked by the character of the growth medium. But such an abstruse supposition seems unnecessary in view of the fact that most growth media, as ordinarily prepared, contain substances (*speciamines*) which have profound effects on pollen, or the tubes produced in presence of them.

Against the suggestion ascribing mutation primarily to change in sap composition, it may be objected that the observed changes in character of the pollen tubes were of the nature of monstrosities. But innumerable gradations were observed between the ordinary tubes and the extreme abnormal types. And, moreover, to attempt to draw a clear distinction between mutants and monstrosities would not seem prudent.

It is believed that, with the knowledge of the disturbing character of foreign sap in an organism, the hope may be entertained of finding the cause of many of the virus diseases of plants and of animals, and mayhap also a means of mitigating these maladies. It is highly probable that many of the virus diseases of plants, which are known to be transmitted by insects, may prove to be

engendered primarily by them with their own secretions or sap from other species on which they had previously sucked. May not even sarcoma in man and other animals be of similar origin?

But until exact data be accumulated concerning the relations of speciamines to mutation, disease, etc., theorising on anything more than general lines would seem unwarranted.

My best thanks are due, and are now tendered, to Mr. Besant, Acting Curator of the Botanic Gardens, Glasnevin, and to Mr. Bennett, Horticultural Instructor at the Dun Laoghaire Technical Institute, for arranging for a supply of some of the plant specimens used in these experiments; and to Mr. Timothy Murphy, for able assistance in the general arrangements.

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No. 41.

LORD MORTON'S QUAGGA-HORSE HYBRID: WAS IT A HYBRID?

By JAMES WILSON, M.A., B.Sc.

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Since Lord Morton wrote[1] about them, in 1820, to the President of the Royal Society, his quagga-horse hybrid and her half-sister and half-brother have been the main supports of the theory that a dam is "infected" by the sire of her first-born young. Lord Morton's letter was as follows:—

"My Dear Sir,

I yesterday had an opportunity of observing a singular fact in natural history, which you may perhaps deem not unworthy of being communicated to the Royal Society.

Some years ago, I was desirous of trying the experiment of domesticating the quagga, and endeavoured to procure some individuals of that species. I obtained a male; but being disappointed of a female, I tried to breed from the male quagga and a young chestnut mare of seven-eighths Arabian blood, and which had never been bred from: the result was the production of a female hybrid, now five years old, and bearing, both in her form and in her colour, very decided indications of her mixed origin. I subsequently parted with the seven-eighths Arabian mare to Sir Gore Ouseley, who has bred from her by a very fine black Arabian horse. I yesterday morning examined the produce, namely, a two years old filly and a year-old colt. They have the character of the Arabian breed as decidedly as can be expected, when fifteen-sixteenths of their blood are Arabian; and they are fine specimens of that breed; but both in their colour, and in the hair of their manes, they have a striking resemblance to the quagga. Their colour is bay, marked more or less like the quagga in a darker tint. Both are distinguished by the dark line along the ridge of the back, the dark stripes across the fore-hand, and the dark bars across the back part of the legs. The stripes across the fore-hand of the colt are confined to the withers, and to the part of the neck next to them; those on the filly cover nearly the whole of the neck and the back, as far as the flanks. The colour of her coat on the neck adjoining to the mane is pale, and approaching to dun, rendering the stripes there more conspicuous than those on the colt. The same pale tint appears in a less degree on the rump; and in this circumstance of the dun tint also she resembles the quagga. The colt and filly were taken from grass for my inspection, and, owing to the present state of their coats, I could not ascertain whether they bear any indications of the spots on the rump, the dark pasterns, or the narrow stripes on the forehead, with which the quagga is marked. They have no appearance of the dark line along the belly, or of the white tufts on the sides of the mane. Both their manes are black; that of

[1] Philosophical Transactions, 1821, p. 20.

the filly is short, stiff, and stands upright, and Sir Gore Ouseley's stud-groom alleged that it never was otherwise. That of the colt is long, but so stiff as to arch upwards, and to hang clear of the sides of the neck; in which circumstance it resembles that of the hybrid. This is the more remarkable, as the manes of the Arabian breed hang lank, and closer to the neck than those of most others. The bars across the legs, both of the hybrid and of the colt and filly, are more strongly defined, and darker than those on the legs of the quagga, which are very slightly marked; and though the hybrid has several quagga marks, which the colt and filly have not, yet the most striking, namely, the stripes on the fore-hand, are fewer and less apparent than those on the colt and filly. These circumstances may appear singular; but I think you will agree with me, that they are trifles compared with the extraordinary fact of so many striking features, which do not belong to the dam, being in two successive instances, communicated through her to the progeny, not only of another sire, who also has them not, but of a sire belonging probably to another species; for such we have very strong reason for supposing the quagga to be.

I am, my dear Sir,

Your faithful humble servant,

MORTON.

Dr. W. H. Wollaston.

P.S.—I have requested Sir Gore Ouseley to send me some specimens of the hair from the manes of the sire, dam, colt, and filly; and I shall write to Scotland for specimens from those of the quagga and of the hybrid.

I am not apt to build hypotheses in a hurry, and have no predilection either for or against the old doctrine of impressions produced by the imagination; but I can hardly suppose that the imagination could pass by the white tufts on the quagga's mane, and attach itself to the coarseness of its hair.

Wimpole Street, August 12th, 1820."

If Lord Morton's description of him be correct, Sir Gore Ouseley's stallion was not the sire of the chestnut mare's second and third foals, nor of a fourth, which was foaled in 1821; for these three foals were all bays, and a chestnut mare cannot have bay foals to a black horse.

There are five horse colours: grey, dun (including cream), bay (including brown), black, and chestnut: and, reading from the left, each is dominant to those on its right, reading from the right, recessive to those on its left. Bay, for example, is dominant to black and chestnut, recessive to dun and grey. Further, if an animal be not pure for the colour it bears, it can carry the factor for one other colour only. The possible combinations of factors in each of the five colours, pure and not pure, are:—

Grey	...	Gr. Gr.;	Gr. dn.;	Gr. by.;	Gr. bl.;	Gr. ch.[1]
Dun	...		Dn. Dn.;	Dn. by.;	Dn. bl.;	Dn. ch.
Bay	...			By. By.;	By. bl.;	By. ch.
Black	...				Bl. Bl.;	Bl. ch.
Chestnut	...					Ch. Ch.

[1] See Wilson, The Inheritance of the Dun Coat Colour in Horses, Sc. Proc. R.D.S., 1912, 13, p. 184; and A Manual of Mendelism.

Since black and chestnut are both recessive to bay, a black with a chestnut cannot have bay foals. If the black be pure, it can have black foals only with chestnut; if it be not pure, but carry both the black and the chestnut factors, it can have both black and chestnut foals.

Sir Gore Ouseley's stallion was not a black, however, but a bay, and, therefore, competent to sire the three bay foals. He was what is frequently misdescribed as black, but was really a very dark bay, which by some might be described as a very dark brown. His portrait in oil by a good artist, Agasse, and those of the other six animals mentioned, are stored in the Museum of the Royal College of Surgeons in London; and Sir Arthur Keith very kindly had them brought to the light for inspection, fourteen or fifteen years ago. The quagga was painted from a drawing, the others from life. The chestnut mare herself is a ruddy chestnut; her first foal, by the quagga, is a ruddy bay; her three foals by Sir Gore Ouseley's stallion are darker bays, of a tint sometimes called mahogany bay.

It may also be mentioned that the paintings indicate no dun tint on any of the chestnut mare's foals.

But was the quagga the sire of the chestnut mare's first foal? Could a chestnut and a quagga have had a bay foal—a foal of a colour dominant to chestnut? The quagga was closely related to the zebra; less closely to the donkey. Zebra and horse foals are sometimes bred, but their colours are one or other of the horse colours, not the zebra colour. At Penicuick, Professor Cossar Ewart bred many zebra-horse hybrids, but, though most of them were striped on the body and barred on the legs, like the chestnut mare's foals, the basic colour of all was one or other of the horse colours transmitted to them by their horse parents. Horse and donkey hybrids are very common, but all are coloured like horses; none is donkey coloured. Thus, the colours of the zebra and the donkey are recessive to the horse colours. So also, presumably, was the colour of the quagga. Therefore, since her own colour was the lowest of the horse colours, the chestnut mare's first foal, had the quagga been its sire, should have been a chestnut. In all probability, only Lord Morton's groom and the leader of some indifferent, light-legged stallion travelling the Lothians in 1814 knew the sire of Lord Morton's hybrid.

No. 42.

A MODIFIED MICRO-METHOD FOR THE DETERMINATION OF MOLECULAR WEIGHTS.

By J. REILLY, M.A., Sc.D., and G. T. PYNE, M.Sc., F.I.C.

[Read June 28. Published separately July 25, 1927.]

In the course of an investigation, still proceeding, on the pigment produced by *Chromo-bacterium violaceum*, it became necessary to make a determination of the molecular weight of the substance. Only very minute amounts of the pigment were available, so that a micro method was essential. The ebullioscopic method of Pregl (Quantitative Organic Micro-Analysis, 2nd edit., Trans. Fyleman, p. 164) was first tried, but was found unsuitable for a variety of reasons—mainly on account of the low solubility of the pigment in the ordinary solvents, and its apparently high molecular weight. Only one solvent—namely, pyridine—was found which could dissolve the pigment in sufficient amounts to affect the boiling point to any extent, but the difficulties and uncertainties attending its use were so great that the attempt was abandoned. The camphor method of Rast (Ber., 55, 1051 (1921)), which has been widely used in micro-work in recent years seemed to offer some prospect of success, as the pigment showed slight solubility in camphor, and was accordingly tried. This method depends on the extraordinarily great freezing point depression constant of camphor (40° C. for one gram molecule of dissolved substance in 1 kg. camphor), which allows the molecular weight of a substance to be determined from a simple observation of the "mixed" melting point of the substance and camphor, taken with an ordinary accurate thermometer. With substances of fair solubility in molten camphor (*e.g.*, 10 per cent.) and average molecular weights, a freezing point depression of about 8° to 20° C. is obtained; but, as has been noted by Pregl (*loc. cit.*, p. 172), the method is obviously limited for many substances by their low solubility in camphor. This was the case with the pigment under investigation. Its solubility in camphor did not much exceed 1 per cent., and this, taking into consideration also its high molecular weight, indicated a maximum freezing point depression of not more than about 0·5° C. Such a small depression could not be accurately measured by the ordinary means. As the camphor method seemed, however, to be the only one which offered any chance of success with the pigment, it was determined to attempt to modify it, so as to enable these small depressions to be estimated with accuracy. The consequence has been the devising of a fairly simple modification of the camphor method, which permits of the determination of molecular weights from quite small depressions of freezing point. Though devised to meet the particular difficulties presented by the pigment under discussion, it appears to be of general application, and, as it may prove serviceable to other workers, it has been thought advisable to publish this note.

The method briefly depends on the preparation of cooling curves for slowly cooling camphor before and after the addition of the substance whose molecular weight is to be determined. As only small amounts of camphor are employed, the thermometer should be small-bulbed. An Anschutz thermometer, graduated in fifths of a degree, and capable of being read by means of a mounted lens to one-fiftieth of a degree centigrade, is quite suitable. The procedure is as follows:—

0·3–0·7 g. camphor (Kahlbaum's, further purified by sublimation) is introduced into a small, fairly strong test-tube (10 mm. × 60 mm.), which is closed

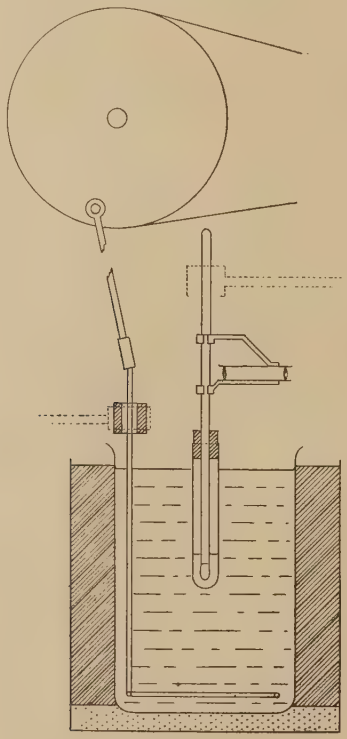

Fig. 1.

by a well-fitting cork, carrying the thermometer. The tube is then immersed up to 2–3 mm. from the bottom of the cork in glycerine contained in a 400 c.c. beaker, which forms the heating bath. It is necessary to completely close the test-tube in this manner, and almost totally immerse it in the bath, in order to prevent sublimation of the heated camphor. The heating beaker is contained in a second vessel (an ordinary tin container serves well), the bottom of which is covered with a layer of sand; and the annular space between the two is filled with loose asbestos. The object of this insulation is to enable cooling to take place sufficiently slowly to give a cooling curve with a well-defined melting point

flattening. A motor-driven stirrer is used, to prevent inequalities of temperature in the glycerine, and thus ensure a perfectly uniform rate of cooling. When the test-tube containing the camphor is in position, the bath is heated to about 180° C. by a Meker burner. As soon as the camphor melts, the thermometer is lowered or adjusted through the cork, so that its bulb is completely covered by the camphor, and centrally positioned. The Meker is then replaced by an ordinary Bunsen or Argand burner, turned down fairly low—the size of the flame being so adjusted that the rate of cooling of the camphor does not exceed 0·8° C. per minute. A rate of 0·4° C.–0·8 C. per minute is the most favourable. The motor stirrer is then set going, and, after the temperature has fallen to 178° C., it is read every quarter of a minute by means of an eyepiece mounted on the thermometer. (See fig. 1.) It is unnecessary to plot the readings to determine the melting point. The temperature is always found to remain steady for two or three readings at some point, which is taken to be the melting point. Sometimes a slight rise in temperature occurs, and then the highest point reached is taken. It is advisable, however, to take readings every quarter of a minute, and not to depend on observation alone to determine the point at which the temperature remains steady, for the latent heat of the small amount of camphor used only produces a very momentary effect on the rate of cooling, which might easily be missed.

While the camphor is still hot, or after it has been remelted if necessary, the thermometer is taken out, allowed to cool, and any camphor which has sublimed around the cork is removed. 2–5 mg. of the substance whose molecular weight is required are compressed to form a small pellet (or a single crystal may be used), weighed on the micro-balance, and dropped into the tube. The stopper and the thermometer are inserted once more, and the tube heated up again in the glycerine bath. As soon as the camphor melts, the test-tube is gently shaken to ensure proper solution of the added substance, the thermometer properly adjusted in the melt, and the cooling curve determined as before. After the estimation, the cork and thermometer are taken out, and the tube is freed from camphor which has sublimed near the cork, and then weighed. The weight of camphor in which the substance was dissolved at the moment of the estimation can thus be determined.

The following are examples of some molecular weight determinations made in this manner:—

Δ = Depression of freezing point.
M. = Molecular weight.
Mol. coeff. of lowering (for camphor) = 40°C. (1 g. mol. in 1 kg.).

1. Acetanilide, 2·54 mg. in 432 mg. camphor.
   Δ = 1.80°C.
   Found M. = 129. Theor. M. = 135.
2. Strychnine, 5·11 mg. in 524 mg. camphor.
   Δ = 1·18°C.
   Found M. = 331. Theor. M. = 334.
3. Palmitic Acid, 5·23 mg. in 689 mg. camphor.
   Δ = 1·18°C.
   Found M. = 257. Theor. M. = 256.
4. *o*-Benzoyl di-*p*-tolyl oxide 3·814 mg. in 293 mg. camphor.
   Δ = 1·66°C.
   Found M. = 314. Theor. M. = 302.

The apparatus described is constructed from the simplest materials. Its use, of course, involves that of a micro-balance, since it is a micro-method. No doubt it could be made still more sensitive, and therefore available for use with even smaller amounts of substances, or those which are less soluble, by some further slight changes. By employing, *e.g.*, a small bulb thermometer graduated in one-tenths of a degree, and a short-focus table cathetometer for reading the temperature, its sensitiveness could easily be increased four-fold. The latter might well prove advantageous in any case, as the existing arrangement of eye-

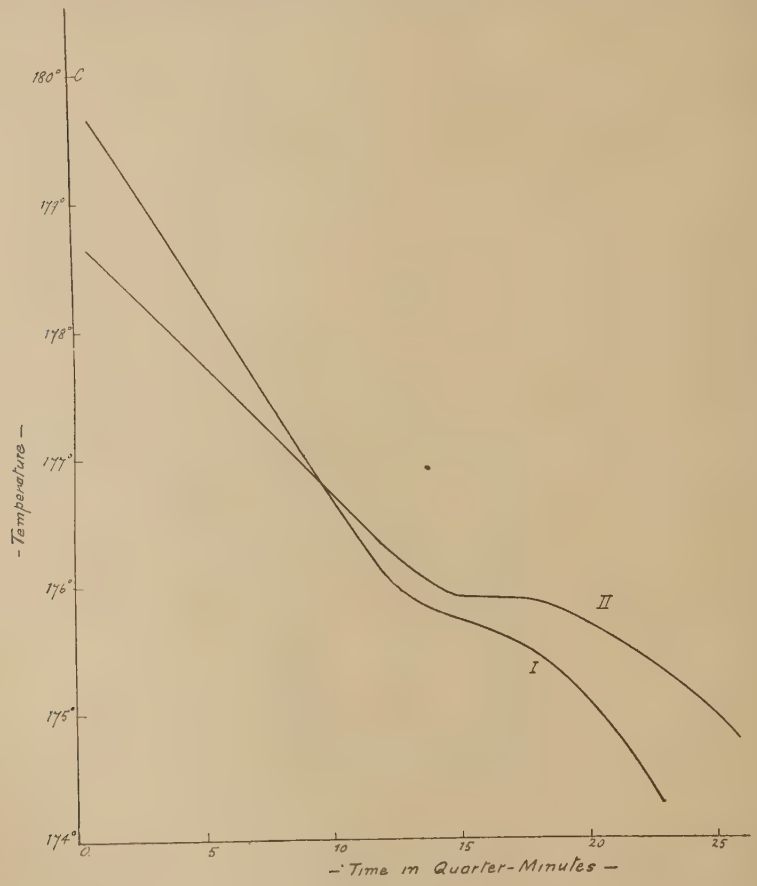

Fig. 2.

piece mounted on thermometer leads to parallax difficulties, even when the lens is moved for each reading, and is liable to cause alterations in the position of the thermometer. Electrical heating might also be used, and would probably give both a quicker heating up and a more easily controlled cooling than gas. Without any of these refinements, however, the method is capable of giving good results, with very little trouble.

The most important manipulative matter is the proper adjustment of the rate of cooling. If this is too rapid, the latent heat evolved on the solidification of the camphor is insufficient to counterbalance it, and a steady fall of temperature ensues, in which no point of arrest can be detected. A rate of fall of about 0·8°, or less, per minute has been found to give satisfactory results. Fig. 2 shows two cooling curves which illustrate this fact. In curve 1 the rate of cooling was about 1·6° C. per minute; in curve 2, 0·85° C. per minute. The second curve shows a marked flattening at one section, which denotes the freezing point of the camphor, while the first shows a continuous fall of temperature without any break. Besides ensuring that the rate of cooling is low enough, the only other point which needs attention is the very obvious, but important, one of inserting the thermometer so that the bulb is completely surrounded by camphor, and equidistant from the walls of the tube. Neglect of this precaution leads to the production of a cooling curve of type 1, in which no clearly defined melting point is discernible.

Summary.

A simple micro-method for the determination of molecular weights is described, which depends on the alterations in the cooling curve of pure camphor produced by minute amounts of dissolved substances. The method is particularly useful for substances whose low solubility in camphor, or high molecular weight, precludes the use of the ordinary Rast method.

University College,
Cork.

No. 43.

COMMENT ON AN ARTICLE BY JAMES WILSON ON "THE MAINTENANCE REQUIREMENTS OF CATTLE ON DIFFERENT RATIONS AND AT DIFFERENT RATES OF PRODUCTION"; WITH A NOTE ON "DYNAMIC ACTION."

By E. B. FORBES,

Director, Institute of Animal Nutrition, Pennsylvania State College.

[Read November 22. Published separately December 5, 1927.]

In the article[1] to which we refer, James Wilson seems to us to have based his computations and conclusions on a seriously erroneous conception; and inasmuch as the work of this Institute is extensively involved, it appears to us necessary to call attention to this error, in the interest of correct general understanding.

Wilson considers the maintenance requirement of energy to include the heat increment—that is, the increase in heat emission, whatever the immediate causes, incident to the taking and utilization of feed. According to Wilson, then, the maintenance requirement of energy is equivalent to, and may be measured by, the total heat emission of the animal, regardless of the plane of nutrition. This conception of maintenance differs from all other standards in that it does not involve nutritive equilibrium, and therefore does not signify *maintenance* in any generally accepted sense.

On this basis Wilson computed the heat emission of cattle, in Kellner's and in Armsby's experiments, to correspond to the uniform live weight of 9 cwts., i.e., 1,008 lbs.

In a personal letter to the writer, Wilson explains that this computation was made by the use of Meeh's formula—that is, the computation of the maintenance requirement from one live weight basis to another, on different planes of nutrition, was made in accord with the two-thirds power of the live weight.

This is clearly erroneous. The basal metabolism, which is measured by the fasting and resting heat production, and which varies approximately with the surface area, may be so treated; as also may be, without extensive error, the maintenance requirement of net energy, which is measured as the 24-hour fasting metabolism with normal activity; but when to this maintenance requirement of net energy is added the extensive energy cost of feed utilization, as in Wilson's so-called "maintenance" requirement, it becomes quite illogical to compute from one to another live weight basis in accord with the two-thirds power of the live weight, because the energy cost of feed utilization is a function, not of the surface of the animal, but, obviously, of the amount of the feed.

All of Wilson's computed "maintenance" requirements, and all other data, graphs, and conclusions based upon these values, therefore, seem to be erroneous.

[1] The maintenance requirements of cattle on different rations and at different rates of production; with a note on "dynamic action." James Wilson. Sci. Proc., Roy. Dublin Soc., xviii, N.S., No. 33, April 26, 1927, pp. 399–406.

No. 44.

THE MAINTENANCE REQUIREMENTS OF CATTLE: A REPLY TO E. B. FORBES' CRITICISM.

By JAMES WILSON, M.A., B.Sc.

[Read November 22. Published separately December 5, 1927.]

Director Forbes is quite correct in saying that my "conception of maintenance differs from all other standards in that it does not involve nutritive equilibrium, and, therefore, does not signify *maintenance* in any generally accepted sense." It was adopted because the conception generally accepted is misleading. Farm stock are not fed at nutritive equilibrium, but generally well above it, and the energy required to manipulate the food, keep the body warm, and produce new tissue—the heat emitted—rises, with acceleration, the harder the animal is fed.

The bulk of Kühn's and Kellner's experimental animals were fed slightly above nutritive equilibrium; the bulk of Armsby's considerably below. From the results obtained with these animals fed as they were, Kellner and Armsby estimated what the animals should have required had they been fed at nutritive equilibrium. Both estimates were wrong: slightly wrong, certainly, but wrong. Then both experimenters said that the food fed to the animal beyond that required for nutritive equilibrium should produce increase in proportion to the quantity fed, no matter what the rate of increase. In this Kellner and Armsby were wrong: very far wrong. I take it that the extra disproportionate quantity of food required, as the rate of increase rises, is for what I have called "maintenance."

Kühn's and Kellner's experimental animals were fed with long fodders only; Armsby's were fed, some with long fodders only, some with long fodders and concentrates. Kellner and Armsby both assumed that the same quantities of metabolisable energy in different foods were of equal benefit to the animal: that, if it required for any purpose, say, 10,000 Calories of metabolisable energy, it was all the same to the animal whether this quantity of metabolisable energy were contained in oat straw, meadow hay, clover hay, or concentrates. In this assumption both Kellner and Armsby were again wrong: excepting that Kellner righted himself to some extent by allowing for the depression caused by fibre.

My diagram endeavours to show how what I have called "maintenance" varies with rate of production, and the kinds and proportions of long fodders in the ration.

Director Forbes says that "in Wilson's so-called maintenance requirements it becomes quite illogical to compute from one to another live weight basis in accord with the two-thirds power of the live weight, because the energy cost of feed utilization is a function, not of the surface of the animal, but, obviously, of the amount of the feed." For this argument something may be said, if the animal's belly be not filled.

Kühn's and Kellner's animals weighed from 11 to 14 cwts.; Armsby's from 3·5 to 8. From the results obtained with these animals both Kellner and Armsby estimated, by the two-thirds power rule, what should be required by animals of standard weights. Thus they also must have been illogical. But were they so very illogical? Does not the amount of food an animal can consume vary with its weight?

No. 45.

CATENARIA ANGUILLULAE AS A PARASITE OF THE OVA OF *FASCIOLA HEPATICA.*

By J. BAYLEY BUTLER, M.A., M.B., B.Ch., B.A.O., Professor of Zoology, University College, Dublin,

AND

J. J. C. BUCKLEY, M.Sc., Research Assistant, University College, Dublin.

[Plates XXIII—XXVI.]

[Read November 22. Published separately December 29, 1927.]

Introduction.

In this paper an account is given of the infection of ova of *Fasciola hepatica* by a Chytridiacean parasite, *Catenaria anguillulae.*

The infection arose in the course of an investigation into the conditions governing the survival of fluke ova when kept in water. It had been suggested that infection of snails by fluke could be greatly reduced if mature fluke were removed from sheep by suitable treatment with antihelminthics at the seasons when miracidia usually hatch.

It is comparatively easy to rid sheep of mature fluke by one or two doses of extract of male fern or carbon tetrachloride, but the young flukes are much harder to remove. From the point of view of the infection of snails, immature flukes are of little importance. This procedure could only be effective if it could be shown that ova laid at other periods of the year did not survive till the normal seasons for hatching.

With a view to determining the length of time that ova could survive in water or moist conditions, a supply of liver-fluke ova was obtained, in January, 1926, through the courtesy of Mr. J. H. Norris, M.R.C.V.S., Director of the Veterinary Research Station of the Department of Agriculture, Saorstát Éireann. The eggs were removed from the bile of an infected sheep, killed at the Veterinary Research Station. The method of removing the eggs was to mix the bile with a large excess of tap water, and allow the eggs to sediment, the supernatant fluid being poured off. After several washings and sedimentations the eggs were obtained free from admixture with bile.

The eggs were kept in test tubes half filled with laboratory water, each containing about 0·5 c.c. of the eggs. The tubes were loosely corked and kept in the laboratory. Periodically, samples of the ova were removed and placed in an incubator at 24° to 26° C.

It was found that, after a minimum period of thirteen days at this temperature, development took place, and miracidia hatched out in large numbers. They continued to hatch subsequently if kept at this temperature.

At intervals of about a fortnight the water in the stock test tubes at laboratory temperature was poured off and replaced by fresh tap water.

Under these conditions the eggs maintained their vitality for a period of nine months. From a sample of the eggs which was placed in the incubator, on the 29th September, 1926, about nine months after they had been obtained from the liver, miracidia hatched out.

Unfortunately it is not possible from this experiment to determine how much longer the ova would have survived, because, on examining some of the eggs in October, 1926, a large proportion of them were found to contain varying numbers of irregularly shaped cysts, which proved to be the sporangia of a Chytridiaccan parasite belonging to the genus Catenaria. The experiment appears to indicate, however, that ova, at whatever period they are laid, survive long enough in water to enable them to live over until the normal hatching period. Should the ova survive similarly under field conditions, as would appear probable, it would scarcely be effective merely to keep down adult flukes at the normal hatching seasons, since there would be a large supply of ova lying dormant where conditions were suitable, which had been laid months previously.

Though the original objective of the experiment was unattainable, owing to the infection which arose, the observations were continued with a view to determining the nature and life-history of the ovum parasite which had thus been unexpectedly encountered.

Source of Infection.

The source of infection of the ova by Catenaria is difficult to determine. The only obvious source of infection, apart from the air or material accidentally introduced by instruments, is tap water, since the ova both at the Veterinary Research Department and the laboratory were washed with water obtained from the Dublin supply.

An attempt was made to ascertain whether the fungus existed in any quantity in the city reservoirs.

We are indebted to Dr. Dwyer, Commissioner, City of Dublin, Mr. R. O. Sullivan, Engineer in charge of the Corporation Waterworks, and Mr. B. G. Fagan, City Analyst, for facilities in examining the reservoir and water supply.

The Dublin city supply comes from an upland catchment area of 14,000 acres, situated at Roundwood, County Wicklow, a distance of 27 miles from the city.

The catchment area is very sparsely populated, and the surface waters from the inhabited districts are diverted by specially constructed channels, and do not enter the reservoirs. The two reservoirs form small lakes, with a combined capacity of 3,700 million gallons.

Within the catchment area large numbers of sheep graze. We are indebted to Mr. Laurence Murphy, Hon. Sec. of the Togher (Roundwood) Agricultural Association, for the information that a few cases of suspected Liver Rot have occurred in the district, but in no case has it been definitely proved that the condition was due to fluke.

Stock is raised in this area, and the store sheep are sent to other districts for fattening, not direct to the abattoir, and so it would be difficult to ascertain whether the disease exists in the district, unless obvious symptoms were presented by the sheep.

From the lower reservoir the water is drawn off at a point approximately 10 feet below the surface, through a valve tower which is not provided with any straining device. It passes into a channel, by means of which it is distributed to the filter beds.

These are of the slow, sand-filter type, and cover five acres. From the filter bed the water passes through an unlined tunnel running through a Cambrian shale rock, to Callow Hill, a distance of two or three miles.

From Callow Hill the water travels by two cast-iron pipes to the service reservoir at Stillorgan. Along the pipe line there are open relief tanks to reduce the pressure. The average velocity of flow is five to six feet per second, and the average time taken by water in passing from Callow Hill to Stillorgan service reservoir is three to four hours.

The service reservoirs at Stillorgan have a capacity of 175 million gallons, or approximately 11 days' supply. Leaving the reservoir the water passes through copper gauze screens, without any further sand filtration.

As a rule, trout are kept in the service reservoirs, but since the summer of 1925, when one of the reservoirs was cleaned, there have been no fish in it. During the summer of 1926, from July to October, a number of small crustaceans, which are described as Daphnia (though they have not been seen by the writers), are reported to have occurred in the pipe supply within the city. They were so numerous as to cause considerable anxiety to the Corporation Engineers, since letters appeared in the local Press complaining of "lobsters" occurring in the water supply.

The nuisance was, to a large extent, reduced by flushing the pipes through the water hydrants in the streets.

From the account above given of the course of the water passing from Roundwood to the city mains, it will be seen that there are two sources of infection from which zoospores could be liberated. In the first place, it is possible that zoospores originating from an alternative host, for example anguillulidae or rotifer eggs, lying on the surface of the sand filter at Roundwood, could reach the service reservoirs at Stillorgan in from 3 to 5 hours. In one of these two reservoirs the outlet and inlet are fairly close together, and it would be possible for the incoming water to reach the mains with comparatively little delay, and so reach the city within a period of 7 hours. In hanging drop preparations zoospores have been seen to remain alive for this period.

Secondly, the large size of the service reservoirs at Stillorgan affords an opportunity for the growth of algal and zooplankton, as will be evidenced by the numbers of crustaceans in the pipes already mentioned, so that zoospores could reach the laboratory tap supply from this source, and indeed also infected rotifer eggs, or other hosts small enough to pass through the gauze strainers on the water towers in these reservoirs. Of these two sources the service reservoirs at Stillorgan seem the more probable.

It appears unlikely that, in this case, the parasite originated from fluke ova hosts, though from which of the possible known other hosts the infection arose, has not been ascertained.

An attempt was made, however, to determine whether the fungus existed in an alternative host in the reservoir at Roundwood. Two nettings were made, both in the main reservoir and in the service channel feeding the filter beds. The plankton material was carefully gone over, but in no case was any material found containing the infection. Small quantities of the plankton material were added to the tubes containing ova obtained under aseptic conditions, but in no case did the parasite appear from such culture.

The examination of the reservoir was by no means exhaustive, so that much reliance cannot be placed on this negative result. Further attempts were made to try and obtain infection by placing small open-mouthed bottles containing ova, killed by boiling, in natural streams and ponds. As will be seen later, dead ova can be infected.

In June, 1927, small bottles containing dead eggs were placed in a stream at St. Fintan's, Howth, County Dublin, and were examined at intervals, but no trace of the infection was found. In this case, too, the experiment is inconclusive, because a considerable amount of sediment occurs in the bottles, making it difficult to recover all the eggs for subsequent examination.

In February, 1927, a tube containing live ova, obtained under aseptic conditions, was placed in a small, artificial, water-lily pond situated in the grounds of Glenlion, Baily, County Dublin. The land around the pond is securely fenced, and the overflow falls direct over the cliffs into the sea, so that there was no danger of spreading infection to sheep or cattle. In this case also the results were negative. These experiments were carried out prior to the identification of the parasite as Catenaria, since, owing to Dr. E. J. Butler's absence in Africa, it was not until August, 1927, he was able to name the fungus for us.

Experiments are now in progress to try and infect eel-worms and rotifers' eggs, and it is proposed to try and infect *Nitella tenuissima,* which Dangeard succeeded in infecting by placing this alga in the vessels containing the infected eel-worms.

When the parasite was first found in October, 1926, it was mistaken for Olpidium. In Dr. E. J. Butler's paper, which is being published on the morphology of this parasite, he states: "Dangeard called attention to the ease with which *C. anguillulae* may be mistaken, when overcrowding or a lack of nutriment causes a reduced development of flask-shaped sporangia, for an Olpidium, and suggests that in some cases *Olpidium endogenum,* A. Braun, might be incorrectly recorded if the rhizoids are overlooked."

Historical account of Catenaria.

The genus Catenaria comprises two species—*C. anguillulae,* Sorokin (8), and *C. pygmaea,* Serbinow (6).

According to Sorokin, *C. anguillulae* is found in Anguillula, encysted infusoria, and eggs of various aquatic "animalculae." It has also been found in *Nitella tenuissima* by Dangeard, who found it in the algal cells when he placed some *N. tenuissima* in the vessels containing infected eel-worms. *Catenaria pygmaea,* if indeed the form described by Serbinow can be included in the genus Catenaria, occurs in the cells of Mougeotiae. In neither case do the eggs of *Fasciola hepatica* appear to have been recorded as a habitat.

Barlow (1) mentions and figures parasites of the ova of *Fasciolopsis buski* (Lankester). He states (p. 81–82): "Freezing kills many eggs, but the greatest number is killed by natural enemies. These consist of diatoms, algae which look like ankistrodesmus and protosiphon (text, fig. XII a and b), and various ciliated protozoa."

From Barlow's drawings, which are reproduced in fig. 45, it seems very probable that the parasites he mentions are not algae, but fungi, possibly allied to Catenaria, if not actually the same species as infects the ova of *Fasciola hepatica.*

The genus Catenaria was established by Sorokin (8) in 1876, when he published an account of the life-history of *C. anguillulae* in the body of the eel-worm, *Anguillula.* He states that the development resembles that of Achylogeton very much. The first phase observed by him was of branching filaments divided by partitions. The filaments became thicker, and later began to swell in parts, forming a chaplet of oblong cells, nearly equally spaced from one another. The cells are joined by very short isthmuses, which themselves

are divided into two by a septum. The cells, or sporangia, produce papillae, or beaks, which pierce the skin of the host. The dimensions of the beaks and sporangia are variable, and he gives the following measurements:—

Sporangia, 10, 15, and 17μ long, and 8 to 10μ wide.

The dimensions of the zoospores given are 1·5 to 2μ.

According to Dangeard (5), 1886, in *C. anguillulae* the following can be distinguished:—

1. A fructifying part formed by filaments swollen here and there into sporangia; these sporangia are usually separated by two sterile cells; the branching is dichotomous.

2. A vegetative part composed of delicate rhizoids, which arise either from the sporangia or from the isthmuses. This, he states, is an absorptive system, very finely branched, and very difficult to perceive.

The zoospores are ellipsoidal with a posterior cilium, and consist of a watery anterior part which encloses little refractive granules. The zoospores germinate by sending out from opposite poles one or more filaments, which extend out to a great length.

Constantineanu (3), 1901, describes sporangia of *C. anguillulae*, the dimensions of which considerably exceed those given by Sorokin. These dimensions are: Length of sporangia, 32–54μ; width, 21–29μ. The beaks also were unusually long, being from 54 to 243μ.

Method of investigation of the Life-History of C. anguillulae in the Ova of F. hepatica.

In order that the parasite might continue propagating, it was, of course, necessary to add fresh ova to the vessels containing the infected ova. We are much indebted to Mr. J. Morris, M.R.C.V.S., Dublin Corporation abattoir, for his courtesy in granting us facilities for obtaining supplies of infected livers, from which the ova were extracted.

To obtain a supply of ova, which were not likely to have been exposed to infection, the gall bladder was removed from the liver, and its surface was washed in a jet of sterilised water. It was then opened with sterilised instruments, and the contents were transferred to a flask of sterilised water, and the eggs were separated in the usual way. As the infection was believed to have originally come through the city water, these precautions were necessary, as the same water supply is used at the abattoir as in the laboratories.

Zoospores.

The zoospores are uniciliate, and more or less oval for the greater part of their free-swimming life (fig. 28 a), but towards the close of this period they become spherical. Amœboid movement (fig. 28 c) is present in some degree, and may be seen during the temporary stoppages, which sometimes occur during this period. During the passage of the zoospore from the sporangium into the dehiscence tube and along the latter, considerable alteration in shape is sometimes necessary. The zoospores (measured after settling on an egg) are about 4μ in diameter (fig. 28 d), and the cilia are between 20 and 30μ long. There is a bunch of highly refractive granules, about six to fifteen in number, situated posteriorly a little to one side of the point of attachment of the cilium, and near the surface of the body of the zoospore (fig. 28 a), and a vacuole occupies a more or less central position. The fixed zoospores (fig. 29) are

roughly 7μ long by 4μ broad, and show the spherical vacuole to be about 3μ in diameter. Attached to the posterior aspect of the vacuole is a conical darkly stained mass, about 1·5μ long. The position occupied by this mass is approximately that occupied by the refractive granules in the living zoospore, and probably this mass represents the aggregated granules.

The zoospores were stained with Heidenhain's iron alum hæmatoxylin, the technique of fixing and staining followed being similar to that described by Curtis (4) for the zoospores of *Synchytrium endobioticum* (p. 436). Instead, however, of picking up the zoospores by suction through a capillary tube, and transferring them to the slide prepared with albumen adhesive as described by Curtis, it was found more convenient, in the present case, to isolate an egg with a sporangium in the act of discharging, and transfer it in a drop of water to the prepared slide.

In swimming the cilium is posterior. The swimming motion is very characteristic, and by reason of this, and by their size, the zoospores can easily be picked out under a low magnification from any other organisms on the slide. The motion is a smooth and steady one, without any zig-zag or rotating action, but the zoospore body is jerked suddenly when direction of movement is being changed, or when the spore comes into contact with some object, and the steady motion is then resumed.

The length of the free-swimming period varies considerably. Zoospores have been observed to cease activity after fifteen minutes; on other occasions they remained active for over seven hours. Those which do not settle on an egg either disintegrate, or lose the cilium and swell up considerably, the contents of the spore appearing to be merged into a single large, highly refractive globule. The rate of swimming is greatest at first, and gradually slows down.

Infection by Zoospores.

In the cases observed, infection of the eggs took place when the zoospores' activity had diminished considerably. The zoospore may approach an egg and alight on it for a second or two, swim off to some other spot on the same egg, and do this several times before settling down permanently. This investigation, as it would appear to be, does not always result in the spore settling down, and swimming activity is continued. The spore may settle on an egg, swim away again, and subsequently die. Not infrequently, dead spores were found in the same drop of water in which uninfected eggs were present and available for attack.

The zoospore, on finally settling down on an egg, is slightly flattened at the point of contact. A membrane is formed around the zoospore, and this remains firmly adhering to the egg, after entry has been effected, retaining the shape of the spore as it first settled on the egg. Other than the formation of this membrane there is no indication of any mechanical device, such as an adpressorium, which would assist in piercing the tough egg-shell, though of course the membrane itself may function in this way.

The perforation itself is not easily visible as long as the spore membrane covers it, and the sporangium forms a more or less opaque background, but if some eggs with empty sporangia are shaken up in water, to remove the spore membranes, and mounted in balsam, the perforations can be seen very clearly. They are usually about 0·5μ in diameter, and, in the majority of cases, are not at right angles to the egg-shell, but pass through it obliquely as in fig. 2. It has not yet been found what time elapses between the settling down of the zoospore and its entry into the egg, but, on one occasion, a zoospore settled

at 11.45 a.m., and entry had not been effected at 3.55 p.m., when it became obscured, and further observation was impossible. During this period the granular material in the spore was in almost continuous motion.

Infection of Dead Eggs.

To determine whether dead eggs could be infected, some eggs, obtained under aseptic conditions, were boiled for five minutes. They were then put in a test tube half full of cold water, and into this was lowered a tube, about 5 cm. by 1 cm., containing infected eggs. This tube was suspended with thread above the bottom of the test tube, but below the level of the water in the latter. After about ten days it was found that many of the dead eggs had become infected, and contained mature sporangia.

Another method was adopted for demonstrating the infection of killed ova. Some fresh ova were boiled for five minutes, and were stained lightly with methylene blue. These blue-stained ova were mixed with living infected ova, and were kept under observation in hanging-drop preparations. It was found that after a week a number of the stained ova contained typical sporangia.

It is clear, from these two observations, that the infection is capable of attacking both living and recently killed ova.

Though a very large number of ova have been kept under observation, in no case has an infected ovum developed into a miracidium.

Development of a Sporangium.

On another occasion (13th April, 1927) a zoospore was observed to settle in a favourable position, namely, at the optical margin of the egg, late in the evening. The zoospore entered during the night, and the next morning there was a cyst about 7μ formed inside the egg, and attached to the shell at the point of entry (fig. 2). The position of the cyst did not correspond with that of the spore membrane, being a little to one side of the latter. Apparently, the penetration of the shell had taken place obliquely.

There were a few granules of different sizes in the young sporangium, the largest of them being about 2μ in diameter, and they were moving slightly. They multiplied with the enlargement of the sporangium, which almost doubled its diameter in about eight hours (figs. 2 to 4).

A very fine process was seen to have arisen from a slightly raised point on the sporangium wall (fig. 4), but could not be found late the next day, and seemed to have disappeared altogether.

From a point on the surface of the sporangium, nearly opposite to its point of attachment to the shell, a mycelial filament had been sent off, but, owing to the density of the yolk at this point, it was not perceived until it had reached the dimensions shown in fig. 5, and had formed a sporangial swelling, a short "isthmus" separating it from the first sporangium. Later, two septa were formed (fig. 7) near the ends of the isthmus, the latter thus becoming an empty cell, separating the two sporangia. Vacuoles appeared in the sporangium—a large one, and one or two smaller ones (fig. 7). These seemed to coalesce, forming a single large vacuole, occupying the centre of the sporangium (fig. 8). The next day the vacuole had almost disappeared, and the granules were becoming smaller, and more numerous (fig. 9). In this figure it can also be seen that the growth of the two sporangia has brought them closer together, and the connecting tube had been compressed up between them. The following day the vacuole had disappeared, and all the granules were small, and evenly distributed throughout the sporangium (fig. 10).

Where the sporangium was in contact with the egg-shell the latter was thinner at one spot, where perforation was taking place prior to the formation of the beak (fig. 10). At this spot the protoplasm was clear, and granules were absent. Some idea as to the rate of growth of the beak can be obtained from figs. 12 to 14, which were drawn up at intervals of six hours.

The next change observed in the protoplasm of the sporangium was a concentration of the granules at the periphery, leaving a space in the centre, somewhat more than half the diameter of the sporangium, in which, however, some granular material still remained (fig. 14). In the meantime, the protoplasm in the lower half of the beak became distinctly vacuolar, and the granules began to get more numerous (fig. 15). During the next few days a second beak was formed at the base of the first, almost at right angles to it (fig. 16). In fig. 13 a tubular process is seen arising from the surface of the sporangium. This structure is a rhizoid. It is characteristic of these processes that, even when they are not obscured by the yolk of the egg, their terminations are very vague and indeterminate. Owing to their transparency and extremely delicate nature, they may easily be passed over, and in the present instance the first appearance of the tube was not noticed. It is probable that it was present from a very much earlier period than is represented in fig. 13.

On the morning of the 21st the granules began to be distributed through the whole of the sporangium (fig. 17), but formed fairly definite groups, each group being composed of from about six to fifteen granules. These groups represent, of course, the developing zoospores. At about the same time the protoplasm of both beaks was vacuolar; later, these vacuoles disappeared, and the granules became grouped, as in the sporangium, the protoplasm of the beaks thus partaking in zoospore formation. At 4.30 p.m. on the same day zoospore formation was complete (fig. 18), and activity began within the sporangium. The apex of the shorter beak alone ruptured, and the zoospores that had formed in the longer beak escaped through the short one. The zoospores leaving the sporangium passed up the long beak first, as this was obviously the easiest path open to them, but, being stopped at the end of this, turned about, passed back along the beak, and escaped through the other. Thus, it came about that in the longer beak were zoospores moving in both directions—some moving towards the apex, and the others retracing their path back to its base, to turn off into the shorter one. This rate of movement in both beaks was very slow, and in the sporangium the spores were unusually sluggish. The sporangium to which the present one was connected matured earlier, and discharged its zoospores two days previously.

The Sporangium.

The dimensions of the fully grown sporangia vary considerably. The largest one met with was 130μ long, and its greatest visible width was 50μ; this was an exceptionally large specimen. In the other extreme, sporangia of about 15μ average diameter are not uncommon. The number of sporangia in each egg is also very variable. Twenty-nine have been counted in a single egg; but this is exceptional, and the average is probably to be found between six and twelve.

There is much variety in the shapes of the mature sporangia (figs. 33–44), and this is due in a large part to growth phenomena sometimes preceding the formation of the dehiscence tubes. In the above instance (fig. 18) the sporangium is globose, and has a single point of contact with the egg-shell, namely, where the zoospore entered, and this point of contact is also the site

of the penetration of the shell for the formation of the dehiscence tube. The globose form is very common, but other forms frequently occur, which show the variety in shape referred to above. In these types a portion of the wall of the sporangium becomes raised, giving the appearance of a slight swelling, which later becomes larger, and finally appears as a rounded process growing from the sporangium (fig. 26). The protoplasm of the sporangium at first seems to be excluded from the swelling, but subsequently invades it (fig. 26 a).

The process continues growing, and eventually comes in contact with the surface of the egg-shell. It may stop growing at this stage, the egg-shell being perforated at the point of contact, or growth may continue in another direction. Should the direction of growth be towards the opercular end of the egg, the end of the process pushes it open, and continues its growth outside as the dehiscence tube (fig. 24). Many parasitised eggs have been examined, but only one has been seen in which the operculum, instead of being burst open by the pressure of the sporangial swelling, apparently offered unusual resistance, and was perforated in the same way as the shell itself (fig. 36). Other irregular shapes are formed when a large number of sporangia develop in one part of an egg, and become elongated, as in fig. 42, owing to lack of space. In this case the clustering of the sporangia at one end of the egg was due to the fact that, as frequently occurs in certain conditions, a large number of zoospores had settled down on one end of the egg, and had entered, each forming a sporangium.

The time taken for the development of the sporangium, or the period between the settling down of a zoospore on the host and the discharge of zoospores from the resulting sporangium, was not found to be less than four days. To investigate this, uninfected eggs were placed in a test tube, and exposed to attack by zoospores from infected eggs. Samples from the uninfected eggs were taken from time to time, and examined for signs of developing sporangia. Another method was by means of hanging-drop preparations. An infected egg was isolated on a cover glass in a drop of water, and a number of fresh eggs was added. The cover glass was then fixed with vaseline to a vulcanite ring, which was cemented to a slide having the centre hollowed out. A small quantity of water was put in the centre to keep the preparation moist. In this way the discharge of zoospores from the infected eggs and the progress of the infection in the other eggs could be followed.

An example of the observations made by this method may be given. An egg containing two ripe sporangia was isolated on a cover glass in a hanging-drop preparation, and forty-two uninfected eggs were introduced. The two sporangia discharged their zoospores on the same day, and four days later one of the fresh eggs was found to have become infected, and contained eight sporangia. Six of these discharged zoospores on the same day, and these zoospores infected ten other eggs. Six more eggs became infected, and the sporangia matured, but, although the slide was kept under observation for some weeks afterwards, no infection of the remaining eggs took place. Possibly, the growth of other organisms in the drop may have produced conditions adverse to the infective powers of the zoospores. This explanation is rendered more probable by the observation of tubes containing infected ova, since in this case comparatively few uninfected ova can be found.

A curious fact was noted in connection with the above experiment. Zoospores which settled on previously infected eggs were seen in many cases to have settled on, or close to, the parts of the shell which were in process of being penetrated by the sporangia previous to the formation of the beaks (fig. 25).

The above method of mounting the material for examination was used in almost all the observations, and proved satisfactory; and although, of necessity, the hanging-drop was usually nothing more than a bare film of water, yet discharge of zoospores and development of sporangia would take place in slide preparations more than a week old.

The refractive and somewhat granular nature of the yolk of the eggs makes it extremely difficult to observe the earlier stages in the development of the sporangia. Occasionally, however, clear spaces without yolk occur in an egg, or the yolk may be less dense in some parts than in others; if these eggs become infected observation is facilitated, and the more delicate structures, such as the rhizoids, and, if the position is favourable, the young vegetative body with its mycelial filaments, become visible. On a few occasions long, slender filaments have been seen growing from infected eggs through the opercular orifice, and extending a considerable distance outside the egg. Their connection with the sporangia in the egg could not be traced with certainty, however, though it is probable that they were rhizoids.

After the entry of the zoospore it appears as a spherical cyst (the vegetative body, or thallus) attached to the inner surface of the egg-shell. The mycelial filament seems to be produced soon after the entry of the zoospore, for it has been seen in vegetative bodies which were, as yet, only about 4μ in diameter. It was at this stage several times the length of the body itself, and proceeded as a single strand for some distance, and then branched, but the termination of the branches could not be found.

In figs. 19 to 21 a sporangial swelling is seen developing. When first observed the mycelium was already considerably swollen (fig. 19, 4.30 p.m., 7/3/27), and was filled with spherical vacuoles and granular material. The more or less spherical vegetative body from which it arises is at the right-hand end, attached to the shell, and the empty zoospore membrane is seen outside. Half-way along the swelling a rhizoid is seen coming off at right angles. At the left-hand extremity there appeared to be another swelling, but this could not be seen distinctly owing to the development of sporangia from another infection, which obscured it.

Figure 20 was drawn at 8.45 on the same day. The swelling had increased in diameter along more than half its length to about twice that of four hours previously, and a septum was formed at a point about three-fifths its length distant from the vegetative body. To the left of the septum the swelling had undergone no increase, and on the next day this had become empty. The main part of the sporangial swelling (which may now be called the sporangium) had become very large, and was connected with the vegetative body by a very short tube, the isthmus, at each end of which was a septum (fig 21, 8/3/27). The sporangium did not become any larger, but penetrated the shell, and formed a beak, and in due course matured, and discharged zoospores.

It will be noticed that with the growth of two sporangia the isthmus separating them is liable to become obliterated, and, in an egg containing a number of fully-grown sporangia, there may be difficulty in finding the isthmuses. When the zoospores have been discharged, they can usually be found, however, as the membrane of the sporangium is transparent.

The Beak.

Each sporangium does not possess more than one beak, that is to say, it does not perforate the shell in more than one place, for branching sometimes occurs.

Two types may be roughly differentiated. In the one type exit from the egg is effected through the operculum, which is pushed open by the growing beak, which continues its growth outside to a length varying from about 20 to about 500μ. The diameter of the beak is greatest at its origin from the sporangium, and it tapers more or less gradually towards the apex, which is rounded. In the other type the sporangium itself, or some outgrowing part of it (fig. 26 d), perforates the egg-shell, and the beak is formed on the outside. This type, in most instances, has a characteristic shape, which is seen in fig. 37. The basal part in contact with the egg-shell is globose, and distally it tapers away gradually to the usual rounded apex. The length of the beak, in this case, is usually about twice its greatest diameter; its average length is about 20μ, but other forms also occur which resemble the first type with regard to shape and dimensions.

The perforations in the egg-shell are circular, and in diameter average from 3μ to 3·5μ. The largest measured was 5μ and the smallest was about 1μ. The measurements were made on ova (mounted in balsam) containing empty sporangia, from which the dehiscence tubes were broken off, or, for some other reason, were missing, so that an uninterrupted view of the holes was obtained.

Discharge of Zoospores.

The discharge of the zoospores is preceded by great activity within the sporangium. Shortly after the outlines of the zoospores become easily discernible, some of the zoospores begin to move very slowly. The movement is gradually taken up by the rest of them, and, usually, a few seconds later, they are swarming actively in the sporangium. The rupturing of the apex of the beak takes place about this time. In most of the instances observed it occurred when the zoospores were beginning their movement, but it may not occur until after the zoospores have reached their maximum activity.

The rupturing consists of the "blowing out" of the transparent, hemispherical cap which covers the end of the beak (fig. 27). The process takes place, usually, in from fifteen to thirty seconds. The inner surface of the cap, which is ordinarily flat, becomes concave, and this is the first indication that the process is beginning to take place. The double contour of the cap becomes increasingly difficult to see as it blows out, as if it were being dissolved, and finally it disappears entirely, leaving no trace. The zoospores, which are becoming more and more active in the sporangium, now begin to emerge. The bore of the tube is rarely large enough to allow more than one zoospore to come out at a time, but, in spite of this, they escape so rapidly that it is difficult to count them as they come out; they are held together in mucus, and literally may be said to pour out through the beak. They form a ball outside the apex of the beak of about 20μ in diameter, but this quickly breaks up, and they swim away. Each subsequent zoospore, after a moment's pause outside, while the cilium is freeing itself from the beak, swims away immediately. Owing to the fact that the opening in the egg-shell through which the beak is formed is smaller in diameter than the width of the zoospores, a slight struggle occurs at this point when the zoospore is passing through.

The body of the zoospore passes down the beak, and is followed by the cilium, which is in motion.

The rate at which the zoospores escape is, in most cases, greatest in the beginning, and decreases as the sporangium becomes emptier. For example, on one occasion 120 emerged in three minutes; at the end of four minutes this

number was increased to 140, and after six minutes to 160. On another occasion the rate of emergence was as follows:—

20	zoospores	in	2	mins.
30	„	„	4	„
34	„	„	6	„
37	„	„	8	„
40	„	„	10	„

It often happens that the last two or three zoospores left in the sporangium have some difficulty in finding their way out, and remain swimming about for a long time before they chance upon the opening, or they may fail altogether to escape.

The average rate of emergence of the zoospores varies considerably in different sporangia; in some it is very high, and in others quite low, irrespective of the size of the sporangia. In some cases the active stage in the sporangia occurred, continued for some time, and then ceased altogether, but no zoospores were discharged. Possibly the exit hole through the egg-shells in such cases was not large enough for the zoospores to pass through. It was not found that the zoospores could remain intact in a sporangium for long periods, and then be discharged, as Curtis (4) describes for Synchytrium. If they are not discharged within a few hours after their formation they die.

One curious observation is worth noting. An egg containing a sporangium in the process of discharging zoospores was isolated in a hanging-drop, containing a very small quantity of water. It happened that during manipulation a small bubble of air passed up the beak, and entered the sporangium. Subsequently, the bubble was expelled, and it was noticed that the zoospores in the sporangium had become considerably more active, and were escaping at about twice their previous rate. This suggests, firstly, that the rate of emergence is—partly at any rate—dependent upon the activity of the zoospores within the sporangium; and, secondly, that the activity of the zoospores may be influenced by the amount of oxygen present.

The chief differences from *C. anguillulae* that the fluke egg parasite presents appear to be in the matter of dimensions and habitat. The greatest size of sporangia previously recorded for *C. anguillulae* is that of Constantineanu, who gave the dimensions 54μ by 29μ, while in the fluke egg parasite sporangia of over 100μ long by 50μ in width are of frequent occurrence.

With regard to the zoospores, in Sorokin's paper (8) they are stated to be 1·5 to 2μ in diameter. These are very much smaller than the zoospores of the fluke fungus, which vary from 6 to 7μ in length, by 4 to 5μ in breadth. Dr. E. J. Butler states in his paper that the zoospores of the fungus described by Dangeard are, according to the figures, up to about 5·5 by 3·4μ.

Summary.

1. The occurrence of a Chytridiacean parasite, *Catenaria anguillulae*, in the ova of *Fasciola hepatica* is recorded. The parasite occurred in test tubes of ova which had been kept in laboratory tap water for some months, and which were still living. The parasite differs from the description of *C. anguillulae* given by Sorokin, Dangeard, and Constantineanu in respect of the larger dimensions of its sporangia, and in respect of the habitat.

2. The source of the parasite is believed to be the tap water in which the eggs were kept. The water supply is derived from the reservoir at Roundwood, Co. Wicklow, a distance of 27 miles from the city. Assuming that the

zoospores were discharged from the original host or hosts either in the reservoir at Roundwood or in the service reservoir at Stillorgan, the time taken for the water to travel from either of these places to the city is not greater than the duration of life of the zoospores, as observed in the laboratory.

3. *Catenaria anguillulae* occurs in the form of cysts, which consist of sporangia, connected together by means of septate isthmuses. In addition, there are nutritive rhizoids. In a single ovum the sporangia vary in number from one to about thirty, and the individual sporangia vary in size from 15μ, average diameter, to about 130μ long by 50μ wide.

4. The sporangia discharge zoospores through a dehiscence tube or beak, which normally pierces the wall of the ovum, and reaches dimensions of between about 20μ to 500μ in length. The beak is cylindrical, usually with a globose enlargement at the base. In some instances the beak pushes open the operculum of the ovum and emerges direct. Only one instance was observed in which the operculum was pierced.

5. The zoospores vary in number between about 40 and 700. Their size varies from 6 to 7μ in length by 4 to 5μ in breadth. They possess a single cilium, a vacuole, and a mass of deeply staining granules. The zoospores remain in a state of activity for a period varying from fifteen minutes to seven hours.

6. Infection of ova takes place by the settlement of a zoospore on the shell of an ovum. The zoospore forms a subspherical cell wall, and pierces the shell of the ovum obliquely, making an aperture of about ·5μ diameter. The penetration takes place within a period of twelve hours from the settling of the zoospore.

7. The development of the sporangium to maturity takes place as follows:—The entered zoospore enlarges, to form a subspherical cyst. A mycelial filament grows out from the cyst into the substance of the ovum, and develops a series of enlargements, which vary considerably in shape. The intervening unswollen parts of the mycelium become septate, and form isthmuses. The sporangia develop nutritive rhizoids, which may branch. Similar rhizoids occasionally occur from the isthmuses. Within the sporangium zoospores develop. The time taken from the entrance of a zoospore till zoospores are liberated is not less than four days, but varies considerably.

8. *C. anguillulae* can be cultivated easily at laboratory temperature in the test tubes containing either living or dead ova in laboratory tap water. A very large proportion of the ova becomes infected.

9. No form of sexual reproduction has been observed.

10. No resting spores have so far been found.

11. The ova of the liver fluke will continue to live and hatch out into miracidia if kept at laboratory temperature for a period of nine months. At any time during this period miracidia can be hatched out by removing ova and placing them in test tubes of water in an incubator kept at a temperature of 24° to 26° C.

12. In no case was it found possible to induce eggs infected by *C. anguillulae* to develop into miracidia.

The possibility of using *C. anguillulae* as a means of checking the infection of snails by miracidia is suggested, and further work on an attempt to culture the fungus in bulk is being undertaken.

Acknowledgment.

We desire to acknowledge very considerable assistance given to us by Dr. E. J. Butler, C.I.E., D.Sc., F.R.S., who has been kind enough to identify the fungus for us, and also to forward us the paper he proposes to publish on the subject

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DESCRIPTION OF DRAWINGS.

Fig. 1. (Pl. XXIII.) 12/4/27. Zoospore having settled on egg and lost its cilium. (Egg-shell is represented by shaded portion.)

Fig. 2. (Pl. XXIII.) 13/4/27. 10 a.m. Shell penetrated, and vegetative body or thallus formed inside. Zoospore membrane adhering to outside of shell.

Fig. 3. (Pl. XXIII.) 13/4/27. 8 p.m. Thallus enlarged.

Fig. 4. (Pl. XXIII.) 13/4/27. 9.15 p.m. A very fine thread-like process arising from surface of thallus; probably a rhizoid.

Fig. 5. (Pl. XXIII.) 14/4/27. 11.30 a.m. Narrow part of thallus becoming visible (previously obscured by yolk of egg).

Fig. 6. (Pl. XXIII.) 14/4/27. 5.0 p.m. Thread-like process disappeared. Septum formed between distal narrow and proximal swollen portion of thallus. Proximal portion now a sporangium.

Fig. 7. (Pl. XXIII.) 14/4/27. 9.0 p.m. Contents of sporangium now becoming vacuolar. Outline of a second sporangium developed from the same thallus now becoming visible. The narrow part of the thallus between the two sporangia, the isthmus, is empty.

Fig. 8. (Pl. XXIII.) 14/4/27. 10.30 p.m. A single large vacuole now replaces the previous two smaller ones.

Fig. 9. (Pl. XXIII.) 15/4/27. 1.0 p.m. The granular material becoming finer, and the vacuole disappearing.

Fig. 10. (Pl. XXIII.) 16/4/27. 12.0 noon. Granules small, and evenly distributed in the sporangium. Perforation of the egg-shell has begun.

Figs. 11, 12, 13, 14. (Pl. XXIII.) 17/4/27. Showing the progress of growth of the beak. Figs. 12, 13, and 14 drawn at 12.0 noon, 6.30 p.m., and 12.0 midnight respectively.

Fig. 15. (Pl. XXIII.) 18/4/27. Granular material in sporangium slightly concentrated at periphery. Contents of tube vacuolar with granules becoming more numerous.

Fig. 16. (Pl. XXIII.)) 20/4/27. A second tube forming at the base of the first, and at right angles to it.

Fig. 17. (Pl. XXIII.) 21/4/27. Granules in sporangium forming groups—the developing zoospores.

Fig. 18. (Pl. XXIII.) 21/4/27. 4.30 p.m. Zoospore formation complete.

Fig. 19. (Pl. XXVI.) 7/3/27. 4.30 p.m. Egg containing a thallus developing two clearly visible sporangial swellings. (Other developing sporangia in the egg, less clearly visible, are indicated by a lighter outline.) The original part of the thallus is globular, and touching the egg-shell. At this point on the outside the empty zoospore membrane is adhering. About half-way along the tubular part of the thallus a rhizoid occurs.

Fig. 20. (Pl. XXVI.) 7/3/27. 8.30 p.m. A septum has formed in the tubular part of the thallus, and to the right of the septum the thallus has swollen considerably.

Fig. 21. (Pl. XXVI.) 8/3/27. 4 p.m. Both parts of the thallus are now sporangia, connected to one another by a short tube, the isthmus, which is septate at each extremity.

Fig. 22. (Pl. XXIV.) Egg with three sporangia; two connected by an isthmus, the other solitary and empty.

Fig. 23. (Pl. XXIV.) Egg with four sporangia, three of which are connected. The undischarged sporangium has one rhizoid, and a rhizoid arises from the isthmus connecting the two empty sporangia. From the larger sporangium a beak has pushed its way through the operculum of the egg.

Fig. 24. (Pl. XXIV.) Three sporangia connected, two of which have two rhizoids each. The middle sporangium has a beak growing out through the opercular aperture.

Fig. 25. (Pl. XXIV.) Sporangia in process of penetrating the egg-shell for the formation of beaks. Illustrating the tendency of zoospores, when settling on eggs already containing sporangia, to settle at the point where the egg is being penetrated from the inside. (These points are indicated by arcs of circles.)

Fig. 26. (Pl. XXIV.) (a) Portion of sporangial membrane becoming raised from the surface of the sporangium.
(b) Growing towards inner surface of egg-shell, and filling up with protoplasm.
(c) Process in contact with inner surface of egg-shell.
(d) Egg-shell penetrated at point of contact, and beak formed outside.

Fig. 27. (Pl. XXIV.) (a) Beak with hyaline cap about to rupture.
(b) Rupturing commenced. Outline of cap becoming faint.
(c) Rupturing nearly complete. Cap about to disappear.

Fig. 28. (Pl. XXIV.) (a) Zoospore. Normal swimming shape.
(b) Zoospore drawn while moving in the beak.
(c) Zoospore. Amœboid movement.
(d) Zoospore after settling on an egg.

Fig. 29. (Pl. XXIV.) Fixed and stained zoospores.

Fig. 30. (Pl. XXV.) Four undischarged sporangia with beaks formed through perforated egg-shell.

Fig. 31. (Pl. XXV.) Three undischarged sporangia with beaks, and one discharged, showing end of beak ruptured.

Fig. 32. (Pl. XXV.) Two sporangia with beaks growing through the opercular aperture, the operculum having been pushed open, and one sporangium which has perforated the egg-shell but not yet formed a beak.

Fig. 33. (Pl. XXV.) Two sporangia with exceptionally broad beaks.

Fig. 34. (Pl. XXV.) Three sporangia, two of which have discharged.

Fig. 35. (Pl. XXV.) Five sporangia, all undischarged.

Fig. 36. (Pl. XXV.) Sporangium which has perforated the operculum and formed a beak outside.

Fig. 37. (Pl. XXV.) Sporangia with beaks of characteristic shape.

Fig. 38. (Pl. XXV.) Six sporangia, two of which seem to be connected by a short isthmus.

Fig. 39. (Pl. XXV.) Two undischarged sporangia. Operculum missing from egg.
Fig. 40. (Pl. XXV.) Sporangia with curiously shaped beaks.
Fig. 41. (Pl. XXV.) Three sporangia, all of which are connected by isthmuses. A rhizoid is apparent in two of the sporangia.
Fig. 42. (Pl. XXV.) About thirteen discharged sporangia bunched at one end of an egg; all with beaks opening through opercular aperture. Outside are a number of empty zoospore membranes.
Figs. 43 and 44. (Pl. XXVI.) Sporangia with a very marked development of rhizoids. Note the septa between rhizoids and sporangia.
Fig. 45. (Pl. XXVI.) Text figure 12 a and b, from Barlow (1), showing "—— algae which look like ankistrodesmus and protosiphon."

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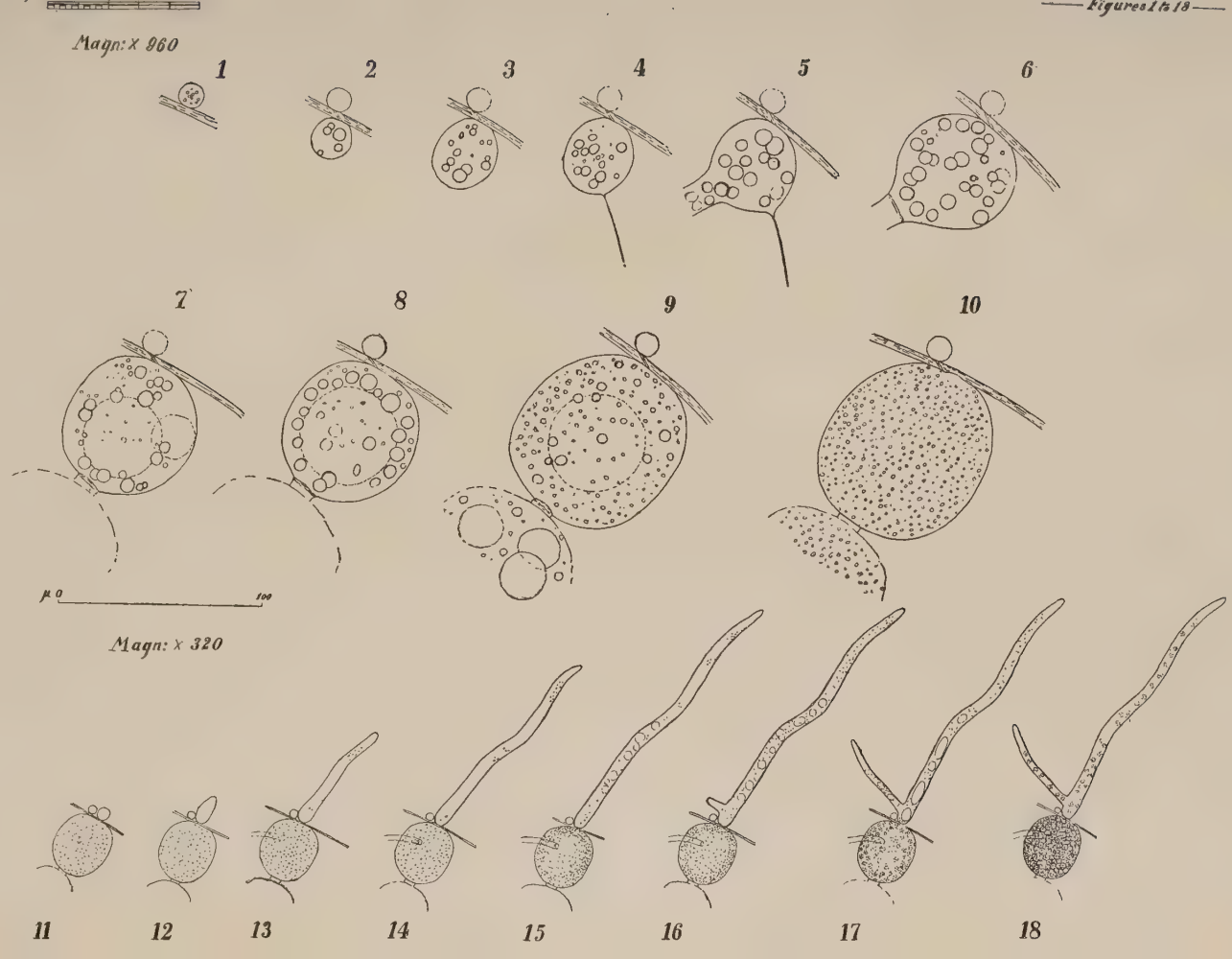
Figures 1 to 18



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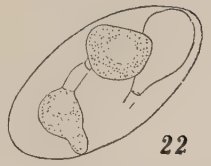
Figures 22 & 29



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a

b

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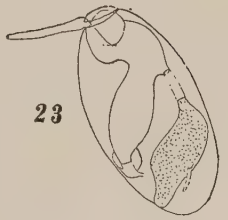

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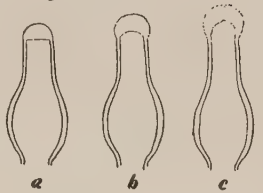

a b c

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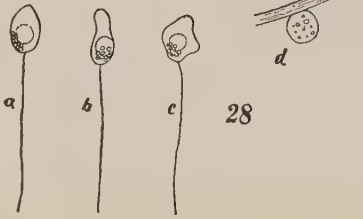

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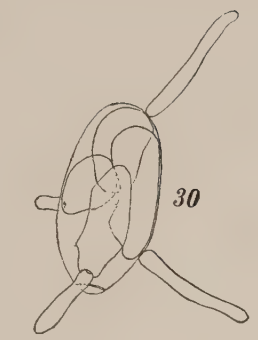
Figures 30 to 42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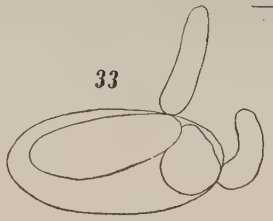
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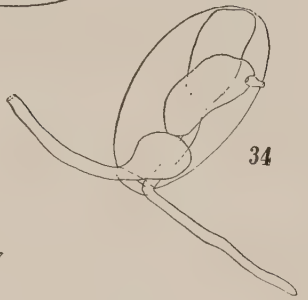
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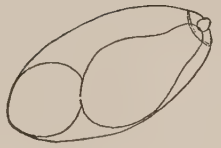
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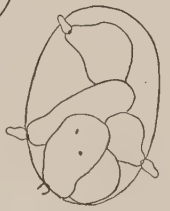
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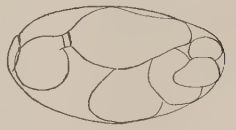
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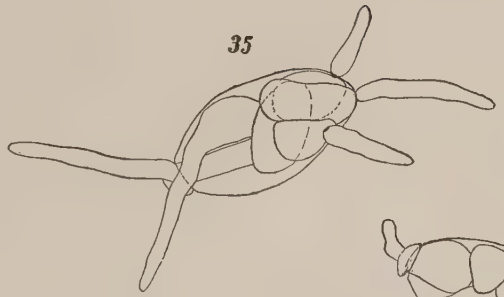
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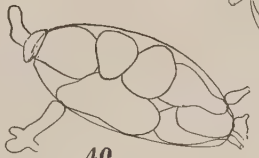
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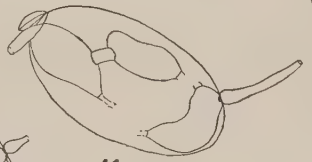
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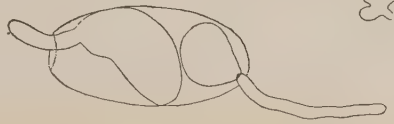
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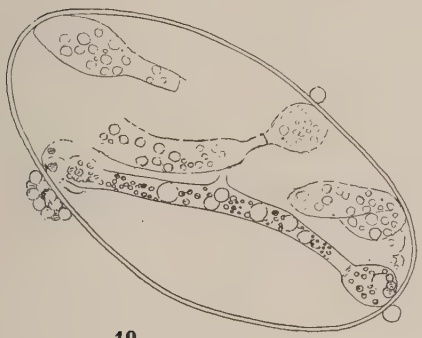

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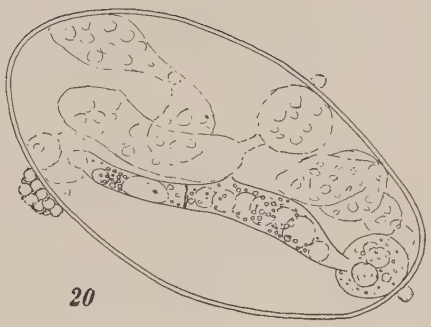

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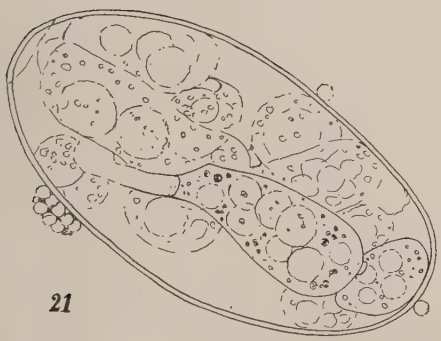

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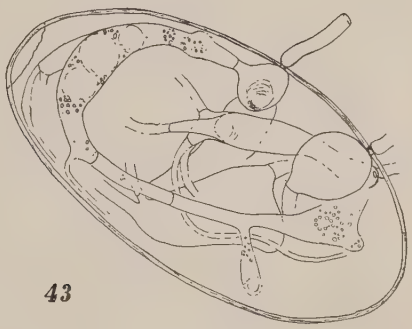

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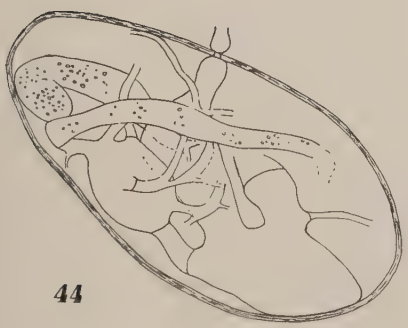

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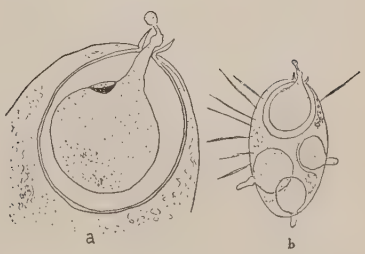

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No. 46.

SOME EXPERIMENTS ON FEEDING RATS WITH SOYA BEANS AND OTHER MATERIALS.

By D. T. BARRY and J. FREUD,

Physiological Laboratory, University College, Cork.

[Communicated by Professor Pringle.]

[Read November 22, 1927. Published separately January 9, 1928.]

These experiments were carried out for the purpose of determining the relative nutritive and biological values of Soya beans and Soya flour. Previous authors generally considered the duration of life of the white rat as an index of the biological value of a foodstuff. Thus, that of Soya bean has been determined by Osborn and Mendel, also that of a flour, produced from it by a special method of Berczeller, has been investigated in a similar way by the latter and his collaborators. The other indicating factor is the change of weight of the animals by exclusive feeding with a certain kind of food.

As the method of determination by the length of life is very long, we have chosen, rather, that of determining changes of weight, combined with occasional estimations of the nitrogen of the urine and fæces. Further, we have measured the quantity of urine and fæces and the gas exchange of the animals in a few cases. We compared the usual mixed diet, consisting of oats and white bread, in equal parts, with (1) Soya flour; (2) Soya beans; (3) meat; (4) white flour; and (5) certain mixtures of these.

Description of the Cages.—These were can-shaped, specially made by a tinsmith, each with a cover, and a funnel opening below a wire gauze floor, on which was the animal. Underneath each was a small bottle with a tin cover, through a hole in which the funnel end was inserted. Natural difficulties in gathering the excreta are due to the habit of the animals of mixing their fæces and urine with food and water, as well as spilling the water. Therefore comparisons over longer periods are necessary, though our food troughs were well designed to obviate these difficulties.

We used tame rats of different colours. All animals were young males. The disadvantages of the cages used were low temperature and lack of full light; but the controls, fed on bread and oats, were kept under exactly similar conditions.

Standard Diet.—Examples for the mixed diet are shown in Chart 1. This shows that feeding with the standard diet keeps the animals rather well in equilibrium, even in the unfavourable conditions of the cage. Graph II in this chart shows even a tendency to increase in weight. Feeding of such animals with meat for periods of 3 days causes marked drop of weight, which sometimes continues to fall for a couple of days after the administration of meat has ceased. The quantities of urine are between 4–6 c.c. per diem. The

first meat period of the first graph gave 10–11 c.c. urine per diem, the second meat period 15 c.c. In the second graph the average quantity of urine in a day is from 6–8 c.c.; during meat Period I it is 20 c.c., and in meat Period II 15 c.c. per diem. The total quantity of food eaten in 72 days is 975 grams, i.e., 13·5 grams per day, for Graph I, Animal I. The quantities taken every day are nearly equal. A slight increase of the appetite is noticeable after meat, the amount reaching an average of 16 grams. For Animal II we have a similar mean for food, and the increased appetite for meat with an average consumption of 23 grams per day. The initial drop of weight, which is very slight, may be explained by a loss of appetite during the first few days in the new conditions

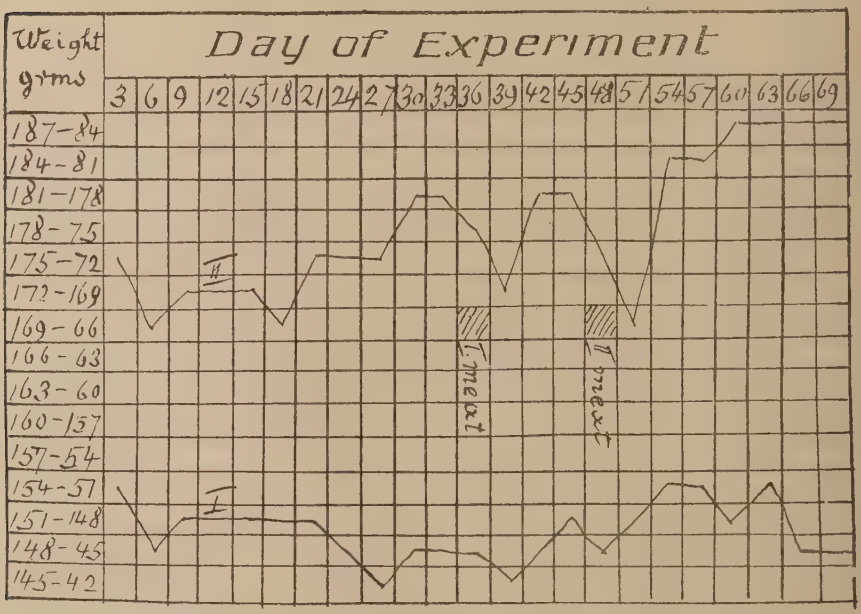


CHART 1.—Two examples of standard diet with two meat periods of 3 days.

of the cages. The consumption of food on those days was below the average. The animals seem to prefer the bread to the oats, because they start by eating the bread. The leavings are chiefly the hulls of oats and the grains, with scarcely ever any bread. The weighing of fæces is rendered very difficult with this standard diet owing to mixture with food in a higher degree than in the other experiments. The quantity varies between 2 and 3 grams per day, though this figure is merely approximative. Kjeldhal estimation gives an average of 1·28 per cent. nitrogen in fæces-urine mixture for the standard diet, and 3·58 per cent. for meat diet, i.e., with regard to the great quantities of urine in such conditions of diet and small quantities of fæces, a large amount of nitrogen passes through the urine. The direct estimations in urine give an average of 1·1 per cent. nitrogen for the standard diet, i.e., here also a greater proportion of nitrogen passes through the urine. We have to account for a

certain discrepancy in the nitrogen values found in the excreta and the nitrogen given in the food. This may be attributed to the fact, mentioned already, that losses of excreta occur in several ways, and also some losses of food. The values given here and in the other series of experiments may not be considered as absolute, but as relative calculations of the total nitrogen metabolism in the different cases. The great variations thus found are beyond doubt an indication of different behaviour of the metabolism with different foodstuffs. The respiratory exchange, in which such errors do not occur, shows an average of 0·0025 c.c. CO_2 and 0·00265 c.c. O_2 per gram per minute. The R.Q. is 0·97, i.e., very high with the mixed diet. The values are reduced to 15° C. and 760 mm. pressure. The quantities given in a day are 30 grams of solid food and 20 c.c. of water, an amount which, compared with the food eaten, shows that the animals had always enough or more, and that changes of weight may not be ascribed to lack of food.

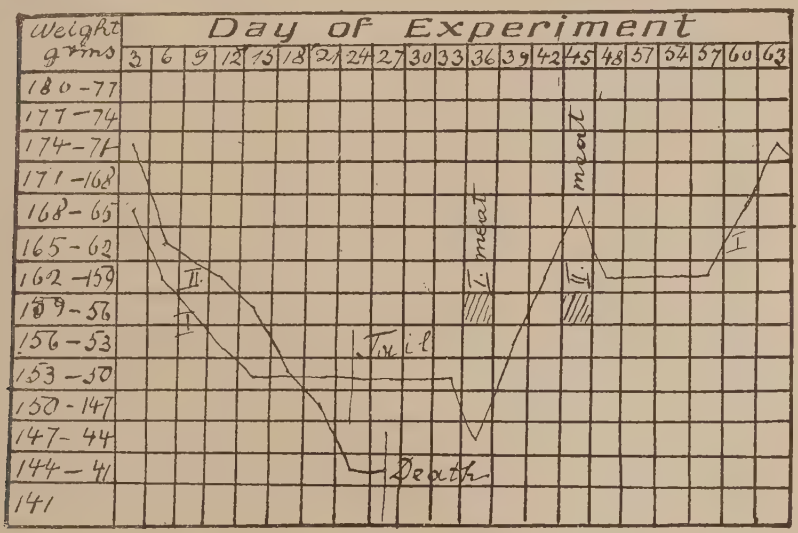


Chart 2.—Two examples of white flour feeding with two meat periods of 3 days each.

White Flour.—The two examples shown in Graphs I and II in Chart 2 are, in spite of equality in external conditions, markedly different from what we saw in Chart 1. Graph I shows the weight of an animal dropping steadily on the exclusive wheat flour diet. The rat started on the 24th day of the experiment to eat its own tail. Later, the drop in weight still continued till the 36th day, when meat was given. This animal took meat more ravenously than any of the others experimented on, and showed, under its influence, a rapid increase of weight, up to the original value. We must consider this experiment as full of interest in all phases. The more usual sequence of events, as also shown in experiments of others, is the constant and rapid decline of the condition of the animal, as shown in Graph II. The pure white flour, as exclusive food, is known to be insufficient. In Graph II the animal died after 27 days. The loss of weight was continuous till death, when it reached about 20 per cent. of the original weight, while in Graph I the loss on this date amounted only to

12·5 per cent. At the post-mortem examination of Animal II, Graph II, no pathological changes were visible; the length of the intestine was found to be 124 cms. The total quantity of food eaten in 27 days (Graph II) was 656 grams, i.e., 24·3 grams per diem. Animal I, Graph I, took 1431 grams of food. The flour was made up with about 20 per cent. water into small balls, so that the dry food was 1145 grams in 66 days, i.e., 17·3 grams per diem. In the meat period (Graph II) the average quantity of food taken is 30 grams. Graph I is,

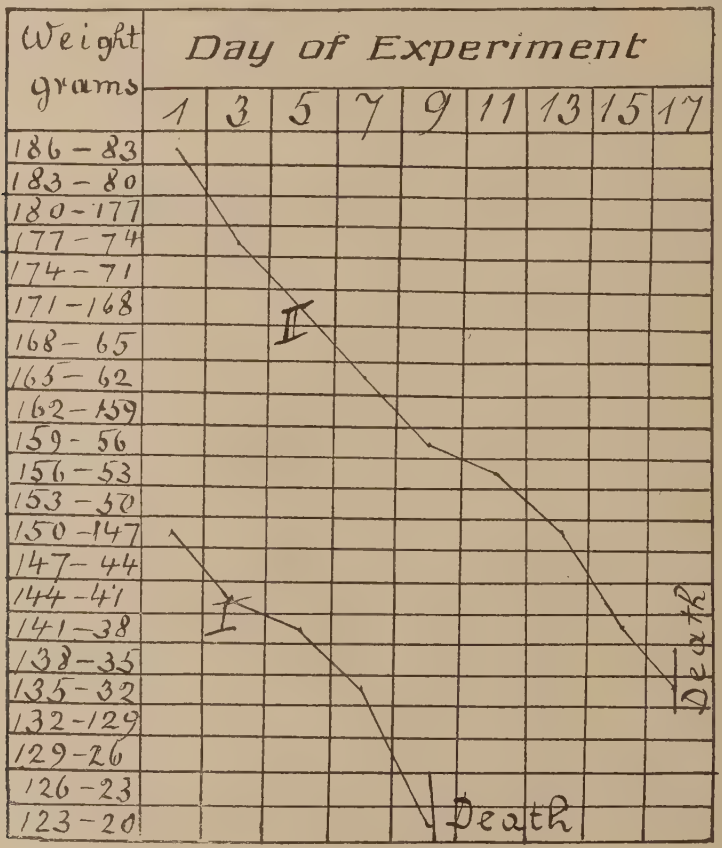


CHART 3.—Showing Soya flour feeding.

in our experience, an extremely rare example of endurance and recovery in exclusive feeding which obviously is biologically insufficient, as shown by the death of Animal II, and the marked drop of weight in Animal I until it started eating its tail. We cannot draw a general conclusion from the single observation, but must state that from our current knowledge of vitamins this fact may not be explained without difficulty. The Kjeldhal estimation of the urine nitrogen gave 1·33 per cent., i.e., slightly higher than in the previously mentioned experiment. Meat increases the value found to 3·1 per cent., i.e., less than after

normal mixed diet. Gas analysis was not done in this experiment. The quantities of food given vary between 30 and 40 grams, corresponding to the appetite. The larger consumption of flour, as compared with bread and oats, is marked. The improvement after feeding with meat in Graph I could be accounted for by restoration of vitamin.

Soya Flour.—Chart 3 shows in two graphs the example of two animals fed purely on Soya flour (Berczeller). The animals in this series generally lost weight rapidly. Death occurred after a loss of 18·6 per cent. of the original weight in Graph I, and of 23·7 per cent. in Graph II. Post-mortem examination showed that the length of the intestine in Case I was 86 cms., in Case II 113 cms., measured from stomach to anus, i.e., shorter than the average in mixed feeding. This observation affirms those of previous authors, and may be considered as a sequence of concentrated protein feeding. Slight hyperæmia of the intestine was shown, with no other apparent cause of death. The heavier animal lived the longer. The quantities of urine are, in both cases, rather variable, between 5 and 10 c.c. per day. The quantity of food taken in 9 days by I was 87 grams, i.e., about 9·5 grams daily, and in Case II 224 grams in 16 days, i.e., 14 grams daily. The quantities of fæces are very large; they vary between 6 and 8 grams, and are of a different aspect and consistence from the others. Obviously we had to do with diarrhœa. The nitrogen estimations—and we again draw attention to the reservations above mentioned—gave 3·36 per cent. for I and 2·51 per cent. for II in the mixture of urine and fæces. The quantities of food given vary from 16–24 grams in a day, according to the appetite.

Chart 4 is a demonstration of the effects of four successive periods of different foods: (*a*) Soya flour, (*b*) white flour, (*c*) white flour and Soya beans (1 : 1), (*d*) the beans alone. The introduction of meat gives a varying result, according to period.

Soya flour alone again causes a drop of weight, so that on the sixteenth day we notice a loss of about 5 to 8 per cent. in Graph I, and the same in Graph II. The initial larger drop again may be accounted for as caused by decreased appetite in the suddenly changed conditions. White flour does not improve considerably the state of the animal; but the introduction of meat in the period of white flour causes a rise in weight, which effect fails 6 days later; a similar result, i.e. a negative effect, occurs in period (*c*). The pure beans keep the animals in fairly good condition for 12 days, but on the fifth day a lowering of their weight begins, which would certainly lead to death in about 3 weeks, as is known from other experiments.

The daily quantity of urine (average for both animals) for the four periods was (*a*) 7–9 c.c., (*b*) 6–8 c.c., (*c*) 12 c.c., and (*d*) 3–5 c.c. The quantity of fæces is again much increased, as well during the feeding on Soya flour as during that with Soya beans. We again met the diarrhœic consistence and light colour of these excreta.

Kjeldhal nitrogen estimations give for period (*a*) 2·24 per cent., for (*c*) 2·3 per cent. After first meat period it is 2·4 per cent., and after third meat period 3·4 per cent. on the average. In period (*d*) we found 2 per cent. nitrogen in the urine. The quantities of food taken average between 15 and 17 grams daily. Slightly increased appetite is noted after each meat period. The animals exhibit, at the beginning of period (*c*), some reluctance towards Soya beans, as compared with white flour, but later on they take them readily.

Respiratory exchange has been determined in the Soya bean period (*d*), and gave as result for:—

	CO_2.	O_2 per gram-minute.	R.Q.
Graph I	0·0041	0·0066	0·61
Graph II	0·0063	0·0098	0·63

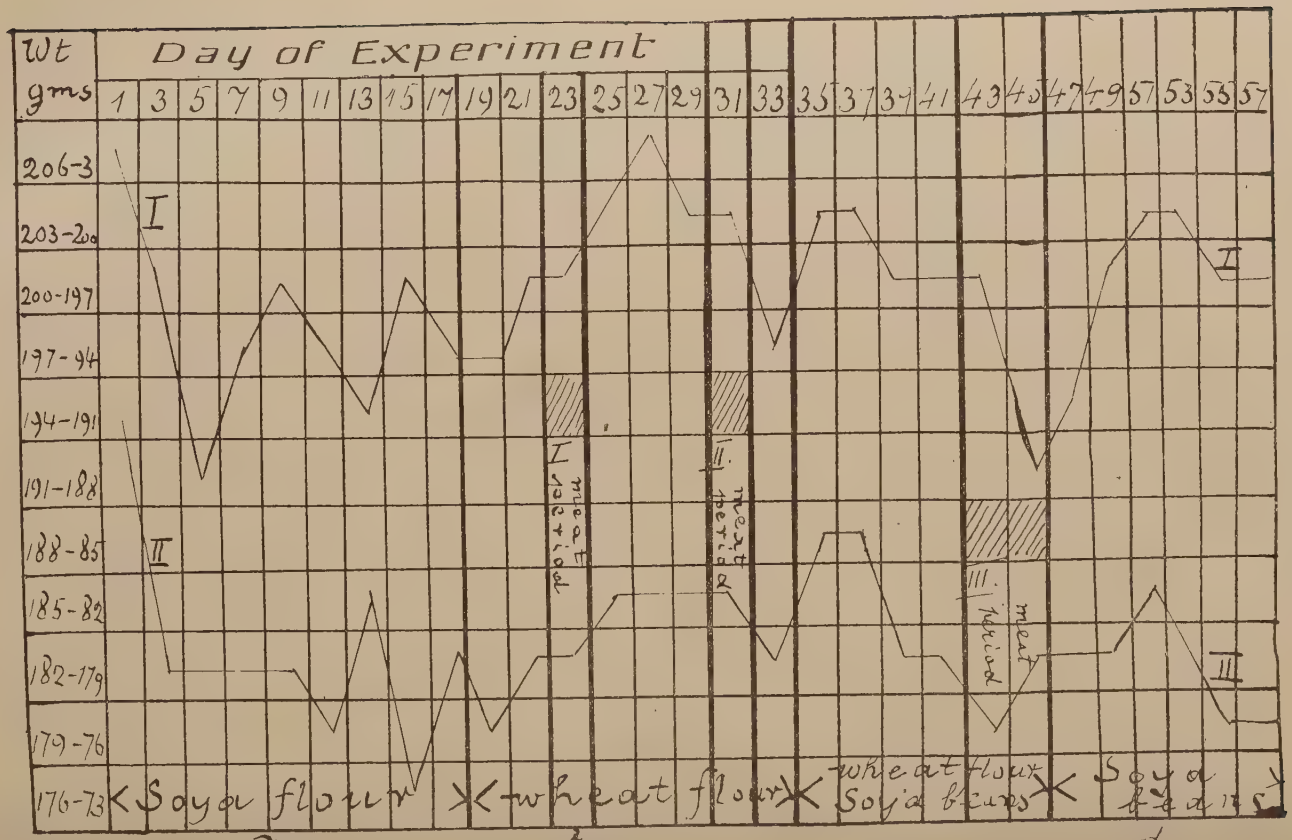


CHART 4.—Showing continued effect of Soya flour, wheat flour, Soya beans, and meat.

The lowering of the respiratory quotient, as compared with that on mixed diet, shows the introduction of considerable quantities of protein into the metabolism; the increased quantities of oxygen used and carbon dioxide produced are obviously due to the specific dynamic action of the same proteins.

The estimation of the respiratory exchange has been carried out with the Haldane apparatus (Eppinger modification). The animals were put into a 2,500 c.c. conical flask, which was connected with the thermobarometer throughout the whole experiment, and thus there was no dead space. The air was thoroughly mixed. One experiment lasted 30 minutes. The quantity of CO_2 did not exceed 1 per cent. at the end of the experiment. The vapours were absorbed by calcium chloride.

Conclusions.—Soya flour alone or beans alone cause loss of weight, chiefly due to the heavy diarrhœa. Meat does not greatly change the condition; therefore there is probably no lack of vitamins.

White flour makes marked protein hunger (tail eaten in experiment, Chart II); while addition of meat greatly improves the condition, probably by vitamin action.

In mixed diet, meat causes loss of weight (specific dynamic action). Here the vitamin supply from meat has no rôle.

Further experiments should be carried out with the Soya products, supplemented by vitamin-free carbohydrates, e.g., starch, so as to see if the nocuous effect is not purely due to lack of carbohydrates and over-feeding with protein and fat (as shown by others).

The comparison of Kjeldhal estimations and respiratory exchange shows that the Soya proteins enter largely into the metabolism, and are of somewhat similar quality to those of meat (*vide* Osborn and Mendel, Berczeller and collaborators).

The general conclusion may be drawn from these admittedly incomplete experiments, that Soya bean and its products are valuable supplementary foodstuffs.

No. 47.

THE FORMATION OF VORTICES BEHIND A CYLINDER MOVING THROUGH A FLUID.

By E. T. S. WALTON.

[Communicated by Professor J. L. Synge.]

(Plate XXVII.)

[Read November 22, 1927. Published separately January 9, 1928.]

Introduction.

It is well known that on the classical hydrodynamical theory for a non-viscous liquid, the resistance experienced by moving bodies should be zero, this result being obviously contrary to experience. The theory has been modified by assuming the presence of surfaces of discontinuity, and this leads to definite values for the resistance in the cases where solutions have been obtained (1). However, it has been found that the theoretical results are not in good agreement with experiment, except for low velocities, as, in practice, turbulent motion is generally produced. If we assume the liquid viscous (and in this case there cannot be a surface of discontinuity), we can obtain the general equations of motion, but these have been solved in only a few cases. Even Stokes' result for a sphere moving with uniform velocity through an infinite liquid is obtained by an approximation, namely, by neglecting the inertia terms in comparison with the frictional terms. Where it is possible to obtain accurate solutions of the differential equations, the steady motion, thus given, is often unstable, and turbulent motion occurs in practice. The nature of this motion, in the two-dimensional case, can be seen readily by merely drawing a stick through some water. In this case vortices are generated alternately on opposite sides of the stick, and these arrange themselves along two parallel lines in an unsymmetrical pattern, as shown in fig. 1.

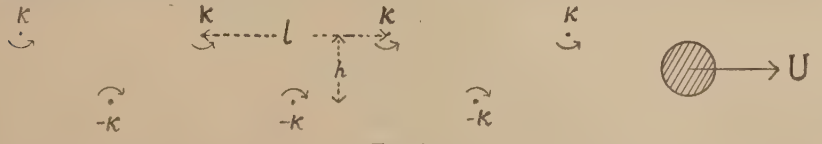


Fig. 1.

Photographs showing the actual generation of these vortices have been obtained by a method due to Professor Hele-Shaw (2), and also by Nayler and Frazer (3). A discussion of the stability of the double rows has been given by Professor Kármán (4), who showed that, in general, the unsymmetric double row is unstable, but that there is neutral equilibrium if

$$\cosh \frac{\pi h}{l} = \sqrt{3} \qquad (1)$$

where h is the distance between the two rows and l is the distance between adjacent vortices on the same row. Hence, if the vortices arrange themselves

on an unsymmetrical double row, we should expect them to satisfy the relation given by the equation

$$\frac{l}{h} = 2{\cdot}74. \tag{1}$$

A photograph taken by Prandtl, agreeing closely with this result, is given in Joukowski's Aérodynamique.

If we assume that this unsymmetrical double row of vortices is formed behind the cylinder, it is possible to derive mathematically several results. If U be the velocity of the cylinder, κ the circulation in each vortex, then it can be shown that the vortices themselves move along in the same direction as the cylinder, with a velocity u given by (5)

$$u = \frac{\kappa}{2l} \tanh \frac{\pi h}{l}. \tag{2}$$

Kármán, by assuming that a surface of discontinuity was first formed, which then broke up into discrete vortices, obtained the equation

$$\kappa = Ul. \tag{3}$$

Using this and the stability condition (*1*), the equation (*2*) yields the result

$$\frac{u}{U} = \frac{1}{\sqrt{6}}. \tag{4}$$

Assuming the above distribution of vortices, Kármán obtained for the resistance per unit length of the cylinder the result

$$R = \frac{\kappa \rho h}{\tau} \tag{5}$$

where ρ is the density of the liquid and τ the periodicity with which the vortices are generated at the cylinder. We also have the obvious relation

$$U - u = \frac{l}{\tau}. \tag{6}$$

Using the equations (*3*), (*4*), (*6*), we may eliminate u, κ, τ from (*5*), and obtain the result

$$R = \frac{\sqrt{6} - 1}{\sqrt{6}} \rho h U^2 \tag{7}$$

$$\text{or} \qquad R = 0{\cdot}592\, \rho h U^2.$$

Kármán found this to be nearly twice that given by experiments on air.

Professor Synge, by a more rigorous analysis, has recently obtained the result (7)

$$R = \frac{\rho \kappa h}{\tau} + \frac{\rho \kappa^2}{2\pi l}\left(1 - \frac{\pi h}{l} \tanh \frac{\pi h}{l}\right). \tag{8}$$

The first term is the same as Kármán's result, and the second term has a value of $0{\cdot}027 \rho U^2 h$, if we assume equations (*1*) and (*3*). This gives for the result

$$R = 0{\cdot}619\, \rho U^2 h, \tag{9}$$

which is slightly further from the experimental result than Kármán's value.

Aim of the Experimental Work.

Experimental work was carried out on the vortices behind a moving cylinder at the suggestion of Professor Synge, in an attempt to discover the reason for the discrepancy between the theoretical formulae and experimental values for the resistance.

To test the various equations used above, the experiments were arranged to give direct measurements of as many as possible of the quantities h, l, τ, u, κ, R, U.

If D is the diameter of the cylinder employed, ν the kinematical viscosity (i.e. ordinary viscosity divided by density), then U, D, ρ, ν may be regarded as independent variables, and the theory of dimensions shows that the six dependent variables are given by equations of the form

$$\frac{h}{D} = f_1\left(\frac{UD}{\nu}\right), \qquad \frac{u}{U} = f_4\left(\frac{UD}{\nu}\right),$$

$$\frac{l}{D} = f_2\left(\frac{UD}{\nu}\right), \qquad \frac{\kappa}{DU} = f_5\left(\frac{UD}{\nu}\right), \tag{10}$$

$$\frac{U\tau}{D} = f_3\left(\frac{UD}{\nu}\right), \qquad \frac{R}{\rho U^2 D} = f_6\left(\frac{UD}{\nu}\right).$$

$\frac{UD}{\nu}$ is a dimensionless quantity, and is generally called the Reynolds' number. A complete experimental solution would give the form of these six functions. $\frac{1}{f_3}$ has been studied by the late Lord Rayleigh (8) and by E. F. Relf (9). The latter used water for low values of the Reynolds' number, and air for the higher values. Relf has also obtained the form of f_6, using air (10).

Up to the present, most of the work done on the vortices generated by a cylinder appears to be confined to the region in the immediate neighbourhood of the cylinder. Professor Synge's proof (7) of his resistance formula merely takes into account the ultimate arrangement of the vortices at some distance from the cylinder, and hence these experiments were designed to give results for as long a train of vortices as possible.

General Description of the Apparatus.

The immersed cylinder was suspended by two knife edges from a carriage, which moved along rails over the tank. It was drawn along by means of a wire, wound round a cylinder, which was rotated by an electric motor. The speed was altered by using a reduction gearing, or by varying the voltage applied to the field, or to the armature, of the motor. The force on the cylinder was measured by the deflection of the suspended part of the carriage. Some aluminium powder was sprinkled on the surface, and the water was blackened with ink powder, in order to show up the vortices well. The vortex pattern was obtained by taking a photograph, and the velocity with which the vortices themselves moved along was obtained by taking another photograph with a second camera after a known interval of time. The cameras were operated by mercury contacts, placed beside the rails, into which a wire attached to the carriage dipped, and thus the exposures could be arranged as desired. The

ratio u/U was simply the ratio of the distances moved by the vortices to the distance moved by the carriage in the interval between the two photographs. These distances were determined by examining the photographs under a travelling microscope. A small flashlight bulb was attached to the carriage, in order to give a definite mark on it. The tank used measured 6 ft. × 1 ft. × 1 ft., this length being found necessary to allow a steady state to be attained during a run of the carriage, and to enable fairly long vortex trains to be studied.

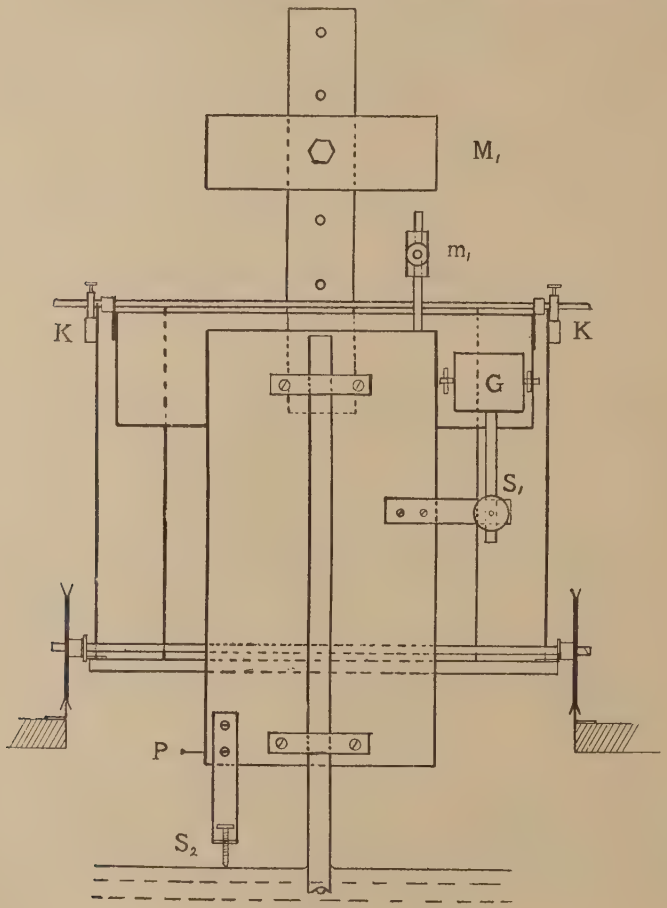


FIG. 2.

The velocity of the carriage was determined by attaching to it a length of morse tape, on which a wavy line was traced by an inked brush attached to an electrically maintained vibrating spring. The period of this was determined by timing a counted number of vibrations with a stop watch. An examination of the tapes showed that the velocity was not quite constant, the variations being generally about 2 per cent., and often as much as 3 per cent. It was found

impossible to get a more uniform speed with the apparatus available. The circulation in the vortices was calculated, using equation (2), namely,

$$v = \frac{\kappa}{2l} \tanh \frac{\pi h}{l}.$$

The only objection against the use of this formula seems to be that, owing to viscosity, the vortices may have diffused, but this should not upset the equation seriously, and values of the circulation thus deduced ought to be fairly reliable.

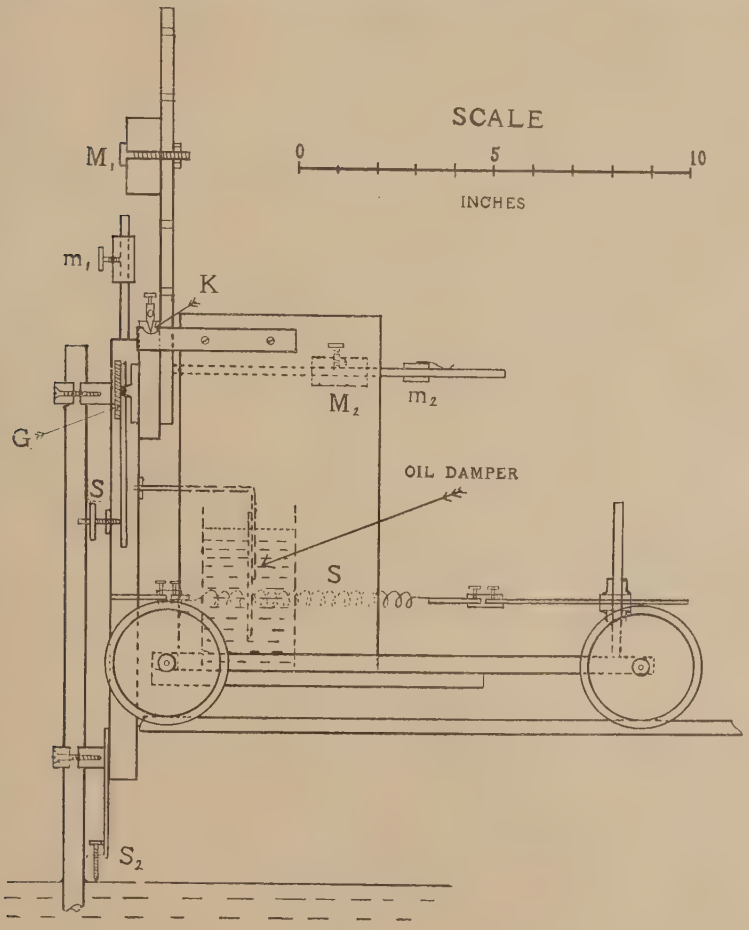


FIG. 3.

The Carriage and the Measurement of Resistance.

An end view of the carriage employed is shown in fig. 2, and a side view in fig. 3. It was mounted on four wheels, giving a wheel base of approximately one foot square, and ran the whole length of the tank on rails, being automatically

stopped at the end. The various cylinders employed could readily be attached to a part of the carriage which was suspended by two knife edges K (see figs. 2 and 3). By altering the position of the weights M_1 and M_2, the centre of gravity of the suspended part can be adjusted so that, when the cylinder is immersed, this part is in neutral equilibrium. The smaller sliding weights m_1 and m_2 are for fine adjustment. When in this state any acceleration of the carriage should have no effect on the inclination of the immersed cylinder.[1] The resistance to the motion of the cylinder was balanced by a spring S, whose tension could be varied, and this was adjusted, so that the cylinder hung vertically when the carriage moved at the speed to be employed during the experiment. This was secured as follows:—A small mirror G was mounted on the suspended part in such a way that it could be rotated about a horizontal axis by turning the screw S_1. Light from a distant source, placed at the same level as the mirror, passed through a lens and, after reflection from the mirror, was brought to a focus on a distant scale, the zero mark of which was also at the same level. The immersed cylinder was clamped in a vertical position, as judged by a small plumb line, which could be attached to the apparatus. The screw S_1 was then adjusted so that the spot of light was brought to the zero mark on the scale. Hence, when the spot of light is at the zero mark, the cylinder is always vertical. The tension of the spring S was then adjusted so that when the carriage was moving at the speed to be employed in the experiment the spot of light was at the zero mark. Thus, the position of the carriage on its rails does not affect the reading of the spot of light, and, owing to the distance of the source and the scale, has only a slight effect on the sharpness of the focus.

In practice, owing to the variations in the speed of the carriage, which caused slight oscillations in the suspended part, the spot of light could not be got to remain perfectly steady on the scale, and an average value was taken for the run of the carriage. In order to diminish these oscillations, an oil damper was employed. It consisted of a flat plate attached to the suspended part, and immersed in an oil bath. To get the numerical value of the resistance, the spot of light was brought to the average reading recorded by deflecting a pendulum bob of known weight, using a thread, which was slipped over the pin P. To get the depth of the cylinder below the surface, the screw S_2 was turned until its point touched the surface of the water. The suspended part was then lifted from the carriage, and the necessary lengths on it measured by a cathetometer.

End Correction.

The theory deals with the two-dimensional case, and hence the cylinder should extend to the bottom of the tank; but this was not considered advisable on account of the large viscous drag that would be produced. This drag would vary greatly if the bottom of the tank were not perfectly flat and parallel to the rails. To allow for the end-effect, the variation of the resistance per unit length of the cylinder was determined while the tank was gradually emptied, and it was found that the variations were no greater than the experimental errors.

Range of the Experiments.

About one hundred photographs were taken, the cylinders used varying in diameter from about 0·6 to 2·6 cm. The lower limit was fixed by the fact

[1] This does not take into consideration the effective increase of inertia of the cylinder due to the presence of the liquid. See Lamb. Hydrodynamics (1924), p. 148. Moreover, owing to the upward thrust of the liquid the apparent centre of gravity of the suspended part is somewhat above the true centre of mass.

that small cylinders gave small vortices, placed so close together that, owing to the manner in which the aluminium powder collected in groups, it was not easy to photograph them. If too large a cylinder were employed, the vortices moved out near the sides of the tank, and thus the conditions under which the theory has been worked out were not satisfied. The velocity of the carriage could be increased up to about 40 cm. per second, higher velocities making the measurement of resistance less certain. However, it was found that, for all the cylinders employed, the vortex train seemed to change in nature well below this figure, and for high velocities very few definite vortices could be seen. Photographs were only taken for the range giving more or less regular vortex trains, but measurements of the resistance were made for higher velocities.

Results.

Three of the photographs taken are shown, as samples, in Pl. XXVII. No. 45 B. shows a cylinder of diameter 2·58 cm. moving at 31·2 cm. per sec., and is irregular. In No. 46 B. the diameter was 2·15 cm. and the velocity 29·4 cm. per sec., and in No. 63 B. the diameter was 0·523 cm. and the velocity 14·9 cm. per sec. Both of these show fairly regular vortices, but those in 63 B. are not as easy to see as in the other two photographs.

Each photograph was examined under a travelling microscope, and the values of l, h, and l/h were calculated for each triad of vortices. It was found that these quantities varied greatly, contrary to what might be expected, judging from the photograph given by Prandtl (11), in which the variations of l/h from the theoretical value 2·74 are very small. At first it was thought that these irregularities might be due to irregularities in the motion of the carriage, but an examination of the photographs and tapes showed that there was no tendency to give a more regular vortex train in the cases where the speed was most nearly uniform. If the irregularities were due to jerks in the motion of the carriage, then, if these jerks were greatly increased in number, we might expect the vortex train to become more regular, for each vortex would be given equal facilities for leaving the cylinder. The experiment was tried, using electric buzzers of different frequencies attached to the suspended part of the carriage, but it was found that no difference was made in the regularity of the vortex train.

In the calculation of the results an average value was taken for l and h, and l/h was taken as the ratio of the average value of l to that of h. When two photographs of the same vortex train were available, an average of both results was taken. The final results were exhibited in the form suggested by Professor Synge, and already described, and graphs were drawn for the six functions f_1, f_2, f_3, f_4, f_5, f_6. In most cases the points showed large variations, as might be expected from the fact that many of them were derived from photographs which were not at all regular. The points were in all cases "smoothed" out by taking an average of them for small ranges of the Reynolds' number, and a smooth curve was drawn so as to represent these points as closely as possible. Points obtained in this way are shown in the graphs by small circles. Nearly all the curves became parallel to the axis for large values of the Reynolds' number, and it is the value of the dependent variable given by this parallel that is of interest, for we are then dealing with the perfect hydrodynamical liquid $\left(\frac{UD}{\nu} = \infty\right)$. The variations of the various quantities with the Reynolds' number were as follows:—

1. *Variation of h/D.*—The variations in h/D were very large, ranging generally from the value 1·3 to 2·7. The "smoothed" points all lay in the

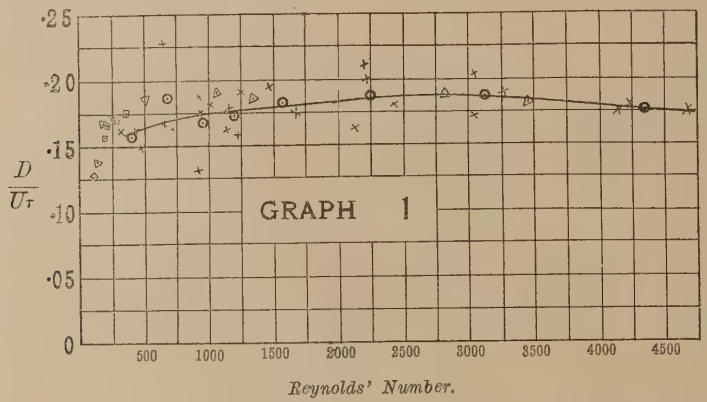
region $h/D = 1{\cdot}75$ to $h/D = 2{\cdot}15$. The curve obtained was nearly a parallel to the axis, showing a slight tendency to rise for small values of the Reynolds' number. The average value obtained for h/D, taking all points into consideration, was 1·92, and the result obtained by using only those points where the Reynolds' number exceeded 1,000 was $h/D = 1{\cdot}86$.

2. *Variation of* l/D.—These variations were not nearly as large as in the preceding case, the values ranging, generally, from about 4 to 5·5. The average values lay very nearly on a straight line, which was inclined at a small angle to the axis, giving a small rise in the value of l/D, with increase in Reynolds' number. The ratio l/D rose in value from 4 to 5 as the Reynolds' number increased from 300 to 4,500.

3. *Variation of* $\frac{D}{U_\tau}$.—The values were calculated, using the equation

$$\frac{D}{U_\tau} = \frac{D}{l}\left(1 - \frac{u}{U}\right),$$

and the results are shown in graph 1. The results obtained by Relf (12) are also shown, his water experiments being given by squares and his air experiments

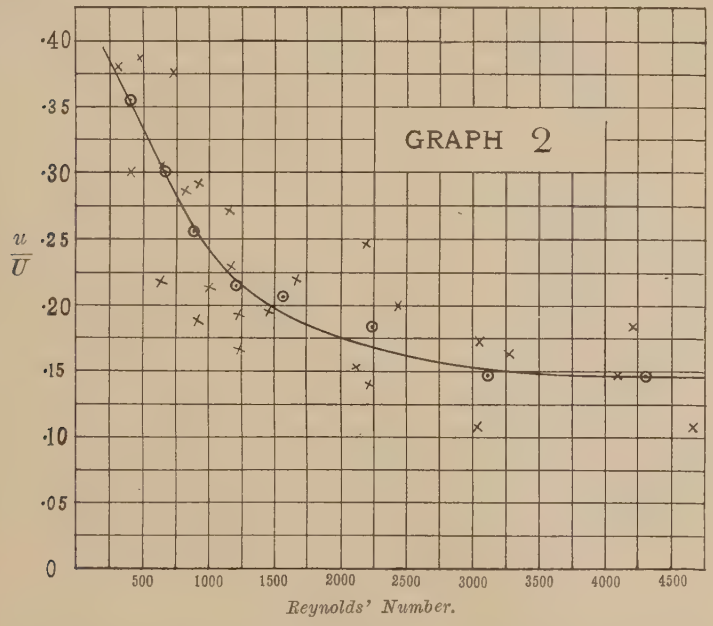


by triangles. It can be seen that these experiments greatly extend the range of his water experiments, and the agreement between his air experiments and these water experiments is good for high values of the Reynolds' number, thus confirming the result predicted from the theory of dimensions. For low values of the Reynolds' number Relf's results are slightly the higher. His method of determining periodicity, being more accurate, gives points which lie better along a curve. To get the value of $\frac{D}{U_\tau}$ for large values of the Reynolds' number, the average value was taken for those points where $\frac{UD}{\nu}$ exceeds 1,250. This gives the result $\frac{D}{U_\tau} = 0{\cdot}185$.

4. *Variations of* u/U.—The results are exhibited in graph 2, and show considerable variations, but the average values (shown in small circles) lie remarkably

well on the curve drawn. This curve shows that u/U decreases rapidly at first from a value of about 0·4 (for the lowest values of the Reynolds' number employed), and tends asymptotically to a value of about 0·15. An average of the six points where $\frac{UD}{\nu}$ exceeds 3,000 gives the result $\frac{u}{U} = 0.146$. This would be the value for a "perfect" liquid, and theory ought to give it. Kármán's theory gives $\frac{u}{U} = \frac{1}{\sqrt{6}} = 0{\cdot}409$, which is a very different result. Professor Synge (7), in a tentative way, suggested the values 0·259 or 0·965, neither of which agrees with these experiments.

5. *Variations of* $\frac{\kappa}{UD}$.—The variations were of roughly the same size as in the case of u/U, and the shape of the curve was also approximately the same, tending to the value $\frac{\kappa}{UD} = 0{\cdot}157$ for large values of the Reynolds' number.

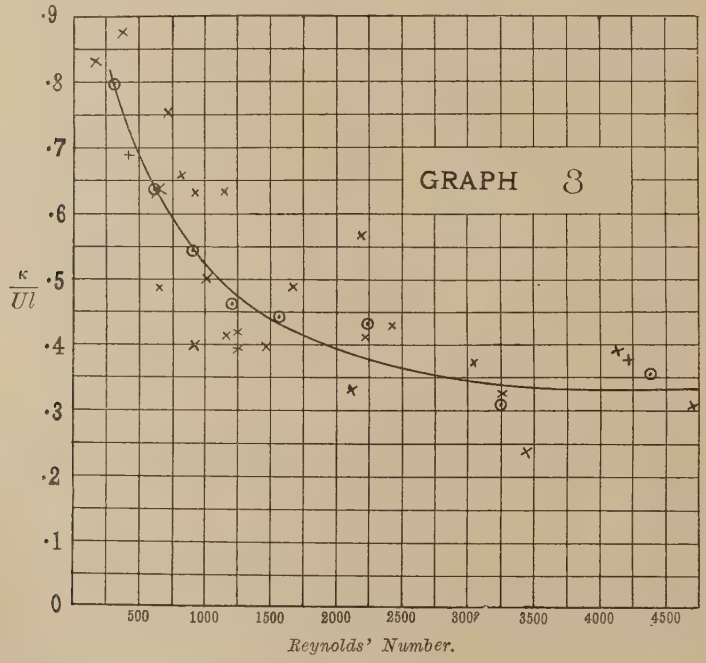


6. *Variation of Resistance.*—The values of $\frac{R}{\rho U^2 D}$ showed greater variations than might be expected from the method of determining the resistance, probably due to the fact that it was not found possible to keep the speed of the carriage absolutely constant. The variations of $\frac{R}{\rho U^2 D}$ are approximately of the same size as those shown by Relf (13) for his experiments in a wind channel. For values of the Reynolds' number between about 2,500 and 4,500 the agreement is quite close, as may be seen from graph 4, where the dotted curve marked R

gives Relf's results, and the continuous curve marked W gives the result of these water experiments. For smaller and for greater values of the Reynolds' number the agreement is not so good, but this may be due to my apparatus, which, possibly, may not yield accurate results for extreme values of the Reynolds' number used. The value of $\frac{R}{\rho U^2 D}$ given by my curve for large values of the Reynolds' number (7,000) may be taken as 0·51; Relf's curve gives 0·55, while in the neighbourhood of the value 28,000 his curve gives the value 0·60.

The approximate agreement between the two curves again confirms a result derived from the theory of dimensions, although the compressibility of air was neglected (14).

7. *Values of l/h.*—These show wide variations, the values ranging from 1 to 4·5. As before, by taking a series of average values, we can get a curve which rises rapidly at first, and gives $l/h = 2{\cdot}93$ for large values of the Reynolds' number. As already remarked, these large variations do not confirm the result given by Prandtl. However, the points on the graph show a tendency to come closer together for large values of the Reynolds' number, and we might hope to get a better result by using larger cylinders and a wider tank. The average value found for l/h, namely, 2·93, is as close as we might expect to the theoretical value 2·74 when the statistical nature of the result is considered.



Comparison of the circulation with that required by Kármán's equation $\kappa = Ul$.

The values of κ/Ul, calculated as already described, were plotted against the Reynolds' number. These points were "smoothed" out, giving the curve in

graph 3. The ratio falls rapidly at first, and tends to a value of about 0·34 for large values of the Reynolds' number. An average of the last six points gave the result 0·334, or just one-third the value necessary to satisfy Kármán's equation. Hence his equation seems to be definitely disproved for the case of chief interest, namely, for large values of the Reynolds' number. The fact that his equation is more nearly satisfied for low values of the Reynolds' number is accounted for by the greater tendency to give a discontinuous motion at low velocities; but in this case the liquid is not so nearly "perfect," and results based on the truth of Kármán's equation may not be valid if we do not take into consideration the effect of viscosity.

Formulae for Resistance.

Kármán's final result was

$$R = 0{\cdot}592\ \rho h U^2,$$

which may be written

$$\frac{R}{\rho U^2 D} = 0{\cdot}592\ \frac{h}{D}.$$

Taking h/D as 1·86, this gives

$$\frac{R}{\rho U^2 D} = 1{\cdot}10,$$

this value being about twice that found by experiment. However, this result was obtained using $\kappa = Ul$, an equation which these experiments have shown to be untrue.

Without assuming the truth of equations (*1*) and (*3*), Kármán's result is

$$R = \frac{\rho \kappa h}{\tau}$$

or

$$\frac{R}{\rho U^2 D} = \frac{\kappa h}{U^2 \tau D} = \left(\frac{\kappa}{UD}\right)\left(\frac{D}{U\tau}\right)\left(\frac{h}{D}\right).$$

Substituting the values found by experiment (for large values of Reynolds' number), we get

$$\frac{R}{\rho U^2 D} = (1{\cdot}57)(0{\cdot}185)\ \frac{h}{D}$$

$$= 0{\cdot}290\ \frac{h}{D}.$$

This is just half the value given by Kármán (namely, 0·592). Substituting now the value found for h/D, we get

$$\frac{R}{\rho U^2 D} = 0{\cdot}539.$$

These experiments gave, for the range 4,000–7,000 of the Reynolds' number, the value 0·51. Relf's value, for this range, ran from 0·50 to 0·55, and, for values of the Reynolds' number in the neighbourhood of 28,000, his value was 0·60. Hence, the observed and calculated values are approximately the same size, provided we do not assume the equation $\kappa = Ul$.

Professor Synge's formula is

$$R = \frac{\rho\kappa h}{\tau} + \frac{\rho\kappa^2}{2\pi l}\left(1 - \frac{\pi h}{l}\tanh\frac{\pi h}{l}\right)$$

or

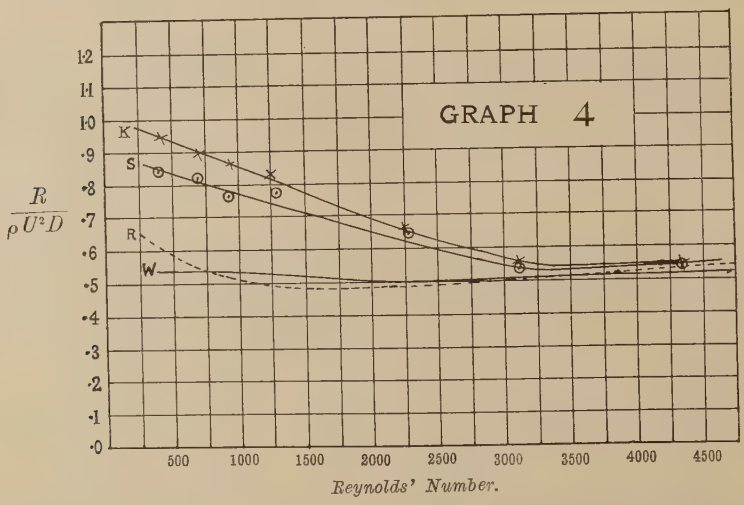
$$\frac{R}{\rho U^2 D} = \frac{\kappa h}{U^2\tau D} + \left(\frac{\kappa}{UD}\right)^2 \frac{D}{2\pi l}\left(1 - \frac{\pi h}{l}\tanh\frac{\pi h}{l}\right).$$

It only differs from Kármán's formula by the second term, which we may call S. The value of l/D was found to rise slowly for the range of the experiments, but, as the term S is only a small correction, we may take the value of l/D as given by the largest values of the Reynolds' number employed. Taking l/D as 5, we get

$$S = (1{\cdot}57)^2 \times \frac{1}{2\pi \times 5}\left(1 - \frac{\pi h}{l}\tanh\frac{\pi h}{l}\right)$$

$$= 0{\cdot}078\left(1 - \frac{\pi h}{l}\tanh\frac{\pi h}{l}\right).$$

It is difficult to know what value to assign to l/h. Taking the theoretical value, namely, 2·74, we get $S = 0{\cdot}005$. Taking the value 2·93, as found from experi-



ment, we get $S = 0{\cdot}012$. In both cases it is a small correction, but its size varies rapidly with the value of l/h. Hence Professor Synge's result is

$$\frac{R}{\rho U^2 D} = 0{\cdot}544 \quad \left(\text{taking } \frac{l}{h} = 2{\cdot}74\right)$$

or

$$\frac{R}{\rho U^2 D} = 0{\cdot}551 \quad \left(\text{taking } \frac{l}{h} = 2{\cdot}93\right).$$

Unfortunately, the experimental results will not distinguish between these two formulae.

The values of $\frac{R}{\rho U^2 D}$ were calculated for each experiment, first assuming Kármán's formula, and then assuming Professor Synge's formula, and, in almost every case, Professor Synge's formula showed a closer agreement with experiment. The points obtained, as in all the graphs, showed considerable variations, and they were "smoothed" out as before. In order to simplify the diagram, only these average points are given in graph 4. The curve given by Professor Kármán's result is marked K, and the points are shown by crosses. Professor Synge's formula gave the curve marked S, and the points are given by small circles. The experimental curve given by Relf (13) is dotted, and marked R, while the remaining curve is that given by these water experiments, and marked W. It can be seen that for low values of the Reynolds' number both formulae deviate seriously from experiment, Professor Synge's giving the better result. For higher values of the Reynolds' number both formulae give approximately the same result, and the two curves approach the experimental ones, showing a fairly close agreement between theory and experiment.

Conclusions.

The theoretical value for l/h was obtained as giving the nearest approach to stability, i.e. neutral equilibrium; and in the proof of this only small quantities of the first order were retained. Hence it is not surprising that the values of l, h, and l/h should show considerable variations, and render all the results of a statistical nature.

The most important deductions from the experiments appear to be:—

(*a*) That the ratio l/h is not constant under the conditions and for the range of these experiments, and tends to give a statistical result which, for large values of the Reynolds' number, is somewhere near the theoretical value 2·74. It is just possible that, by taking great precautions to secure uniformity in the motions of the carriage, the vortex train may become regular and give the theoretical result. But the vortex trains observed in these experiments must remain of chief interest as being what are produced in practical cases.

(*b*) That Kármán's equation $\kappa = Ul$ is not valid except, possibly, for very low values of the Reynolds' number. In this case we are not dealing with a "perfect" liquid, and, consequently, the value derived for the resistance is not reliable.

(*c*) That, if we do not use the equation $\kappa = Ul$, then, for high values of the Reynolds' number, the resistance formulae of Professor Kármán and of Professor Synge are both fairly accurate; while, for low values of the Reynolds' number, both give a value which is much too high, Professor Synge's result being preferable. In the calculation of the latter's result the value of l/h found in the experiment should be used, and not the theoretical result 2·74. The experiments were not sufficiently accurate to distinguish between the two formulae for high values of the Reynolds' number.

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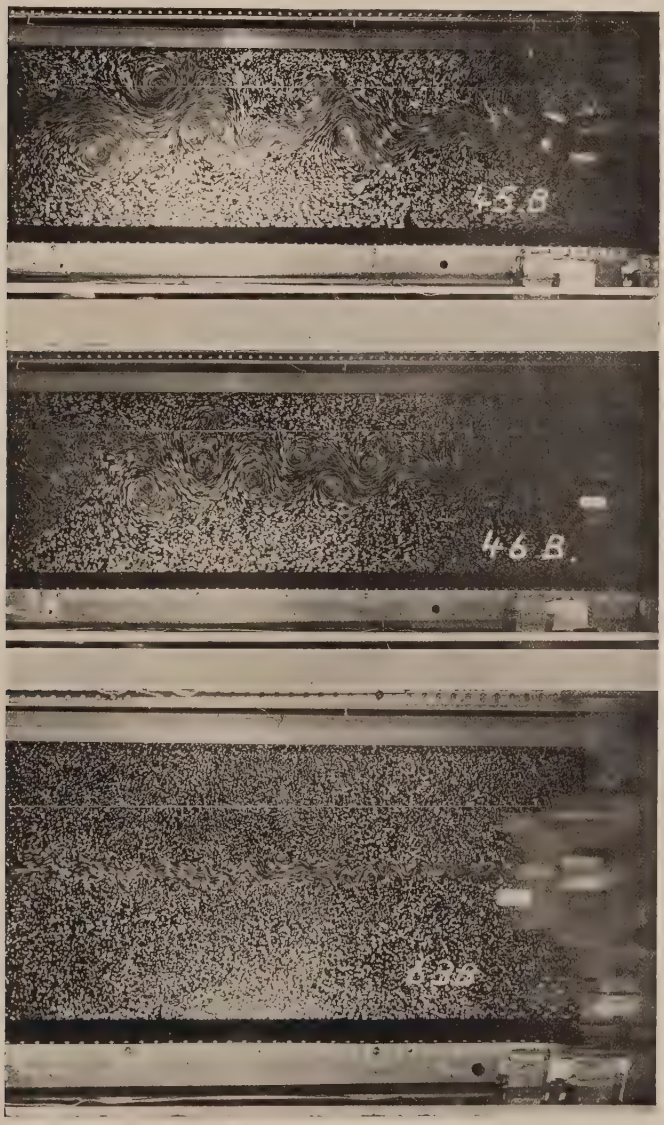
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